"ANTI-HYPERGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF *LANTANA CAMARA* LEAVES"

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Submitted by

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OCTOBER - 2016

Certificates

EVALUATION CERTIFICATE

This is to certify that the work embodied in this dissertation entitled **"ANTI-HYPERGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF *LANTANA CAMARA* LEAVES"** submitted to "The Tamil Nadu Dr. M.G.R. Medical University", Chennai, in partial fulfillment and requirement of university rules and regulation for the award of Degree of **Master of Pharmacy** in **Pharmacology**, is a bonafide work carried out by **Reg.No. 261425225** during the academic year 2015-2016, under the guidance and supervision of **Mr. S.VENKATESH, M.Pharm.,** Assistant Professor, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam.

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CERTIFICATE

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DECLARATION

I hereby declare that the dissertation entitled **"ANTI-HYPERGLYCEMIC AND ANTI-OXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF *LANTANA CAMARA* LEAVES"** has been carried out under the guidance and supervision **Mr. S.VENKATESH, M. Pharm.,** Assistant Professor, Department of Pharmacology, J.K.K. Nattraja College of Pharmacy, Kumarapalayam, in partial fulfillment of the requirements for the award of degree of **Master of Pharmacy** in **Pharmacology** during the academic year 2015-2016.

I further declare that, this work is original and this dissertation has not been submitted previously for the award of any other degree, diploma associateship and fellowship or any other similar title.

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LATHA R

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Dedicated to
Almighty,
My beloved family,
Teachers and friends.

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महोदया/Madam,

The plant specimen brought by you for identification is identified as *Lantana camara* L. var. *aculeata* (L.) Moldenke (= *Lantana aculeata* L.) - VERBENACEAE. The identified specimen is returned herewith for preservation in their college/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

(डॉ. एम. पलनिसामी/Dr. M. Palanisamy)
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1. INTRODUCTION

Traditional Medicine

Traditional medicine (TM) is a very valuable resource because of the long history of its use and thus being evidence based. It is employed extensively in developing countries for primary health care, but of late has aroused increasing interest in developed countries too; 1) as an alternative to high cost medicines for promotive and preventive health care; 2) for disease conditions with inadequate modern drugs and 3) for non-life threatening diseases of lower incidence side effects reported than with modern drugs[1]. WHO defined TM as the sum of the total knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not used in the maintenance of the health and in the prevention, diagnosis, improvement or treatment of physical and mental illness. The major resource base of the TM is medicinal plants with the introduction of modern medicine.

Medicinal plants continued to play a very significant role in the healthcare of human kind. The advancement in modern medicine caused a rapid decline of traditional medicine particularly in developed nations. But medicinal plants continued to meet the health needs of 80% of population in developing countries. Towards the end of the 20th century there began a revival of interest in traditional medicine not only in developing countries, but also in the developed countries. The resurgence of plant based medicine is mainly due to the increasing evidence/ realization of the health hazards associated with harmful side effects of many synthetic medicines and also the hazards associated with the indiscriminate use of modern medicine such as antibiotics, steroids and other synthetic drugs. The increasing popularity in plant based drugs is now felt all over the world leading to a fast growing market for plant based drugs, pharmaceuticals, nutraceuticals, functional foods and even cosmaceuticals[2].

1.1 Indian traditional systems of medicine

The traditional medicine in India functions through two social streams. One is the local folk stream, which is prevalent in rural and tribal villages of India.

The carriers of these traditions are millions of housewives, thousands of traditional birth attendants, bone setters, practitioners skilled in acupressure, eye treatment or treatment of snakebites, the traditional village level herbal physicians (the "Vaidyas") and tribal physicians in the tribal areas. These local health traditions thus represent an autonomous community supported system of health delivery at the village level which runs parallel to the state supported system. A second level of traditional health care system is the academic or classical system. This consists of codified and organized medical wisdom with sophisticated theoretical foundations and philosophical explanations, expressed in thousands of regional manuscripts covering treatises on all branches of medicine and systems like Ayurveda, Siddha, Unani, Yoga, Naturopathy and Amchi, which are expressions of this stream[3].

1.1.1 Ayurvedic system of medicine

Ayurveda is perhaps the oldest among the organized traditional medicine. It spread with *Vedic* and Hindu culture as far in east as Indonesia and to the west it influenced the Greek, who developed a similar form of medicine. The Buddhists added many new insights to it and they took it along with their religion to many different countries. In this way, Ayurveda became the basis of the healing tradition of Tibet, Sri Lanka, Burma and other Buddhist lands and exchanged/ influenced Chinese and Greek medicine. Ayurveda is thus a rich tradition, adaptable to many different times, cultures and climates[4].

Ayurveda originated in India long back in pre-vedic period. Rigveda and Atharva-veda (5000 years B.C.), the earliest documented ancient Indian knowledge have references on health and diseases. Ayurvedic texts like 'Charak Samhita' and 'Sushruta Samhita' were documented around 1000 years B.C. Ayurveda (Ayur: Life; Veda: Science) means science of life in *Sanskrit* and aims at holistic management of health and disease. It deals elaborately with measures for healthful living during the entire span of life and its various phases. Besides, dealing with principles for maintenance of health, it has also developed a wide range of therapeutic measures to combat illness. These principles of positive health and therapeutic measures relate to physical, mental, social and spiritual

welfare of human beings. Thus Ayurveda becomes one of the oldest systems of health care dealing with both the preventive and curative aspects of life in a most comprehensive way and presents a close similarity to the WHO's concept of health propounded in the modern era. In India Ayurveda, Siddha and Unani systems are the formal and most organized ones amongst the traditional systems of medicine. The Tibetan system of medicine is considered as an off-shoot of Ayurveda[5].

Ayurveda remains one of the most ancient medical systems widely practiced in the Indian subcontinent and has a sound philosophical, experiential and experimental basis. *Charak Samhita* and *Sushrut Samhita* (100–500 B.C.) are main Ayurvedic classics, which describe over 700 plants along with their classification, pharmacological and therapeutic properties. Knowledge of Ayurvedic medicine has unfortunately been confined to India and the west is largely ignorant of it. Even in India, this traditional medical practice has lost a lot of its importance in the urban situations. One of the main reasons for this is that much of the early and core medical literature on Ayurveda is in Sanskrit, the ancient language which ceased to be a day-to-day language. Even today a considerable bulk of Ayurvedic knowledge is in the form of ancient palm leaf manuscripts hidden in remote libraries and private collections, and as treasured personal knowledge of a few individuals. The net result is that Ayurveda has been away from limelight and does not enjoy the importance and popularity it deserves.

1.2 Diabetes

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus[6]. Diabetes as a disorder was known and recognized by man since the ancient ages. The first mention of diabetes (though it was evidently not known as "diabetes" then) was found in Indian literature in the works of the physician Susruta (6th century BC), it also finds a mention in Charaka Samhita. The word "diabetes" is derived from a Greek word that means "to siphon or drain off", the

most obvious sign of diabetes being excessive urination. "Mellitus" comes from a Latin word that means "sweet".

Diabetics tend to believe that there is no cure yet for diabetes in any system of medicine anywhere in the world, which is partly because diabetes is not a single disease but is a complex disorder with multiple syndromes making it difficult to cure the cause of the disease. Diabetes is a cluster of symptoms like in an aging process this manifests as graying of hair or wrinkling of skin. As with aging symptoms, diabetes may occur at a very early age in a few, and at some stage of the life in most. Diabetes manifests in different persons in many different ways, and depending on the age, severity of the symptoms and involvement of other organs in the body, medical treatment greatly varies. For example a diabetic, either in young age, during pregnancy (gestational diabetes), if suffering from tuberculosis or a foot ulcer or, with recent heart attack or paralysis, should be given insulin injections only, and not other anti diabetic drugs of any systems of medicine[6].

Diabetes leads to the development of numerous complications due to hyperglycemia. Likelihood of developing complications, whether acute or chronic, is ultimately a reflection of the level of blood sugar control. Diabetics are susceptible top three major complications, hypoglycemia, diabetic ketoacidosis (primarily affects type I diabetics), non ketogenic hyperosmolar syndrome (primarily affects type II diabetics). On a long term basis, the diabetic's health condition is complicated by repeated elevations in blood glucose levels. Five major chronic complications of diabetes are atherosclerosis, diabetic retinopathy, diabetic neuropathy, diabetic nephropathy, and diabetic foot ulcers[7].

Diabetes mellitus is mainly classified as type 1, insulin dependent diabetes mellitus (IDDM) or juvenile-onset diabetes and type 2, non– insulin-dependent diabetes mellitus (NIDDM) or maturity-onset diabetes. Type 1 diabetes is usually associated with rapid onset, mostly in younger people, and is connected in many cases to viral destruction of the beta cells of the pancreas. In type 1 diabetes, there is an absolute deficiency of insulin resulting from autoimmune destruction of β-

cells. Type 1 diabetes always requires insulin replacement. Type 2 diabetes usually occurs in mature adults and has slow and progressive onset. Type 2 diabetes is accompanied both by insulin resistance and by impaired insulin secretion, each of which are important in its pathogenesis. Treatment is initially dietary although oral hypoglycemic drugs usually become necessary, and about one third of patients ultimately require insulin[8].

1.2.1. A rising global burden

Diabetes mellitus (predominantly type 2 diabetes) is a major and growing health problem in almost all the countries. Globally, the prevalence of diabetes in adults aged over 20 years was estimated to be 4% in 1995 and is projected to rise to 5.5% by 2025. The number of people with diabetes will be more than double over the next 25 years, to reach a total of 366 million by 2030. These projections of the number of people with diabetes in 2030 take into account the fact that there will be more people in the world (population growth) and that there will be more elderly people (population ageing). They also take into account trends in urbanization - the fact that people are moving from rural areas to cities, particularly in developing countries. This affects the number of people who are likely to have diabetes, because people living in cities in developing countries tend to be less physically active and have higher levels of overweight and obesity than people in rural areas. In fact, current trends in obesity suggest that these projections are conservative and that the increase in the prevalence of diabetes may be even greater[9]. In 2005, an estimated 1.1 million people died from diabetes. Almost 80% of diabetes deaths occur in low and middle-income countries. Almost half of diabetes deaths occur in people under the age of 70 years; 55% of diabetes deaths are in women[10]. WHO projects those diabetes deaths will increase by more than 50% in the next 10 years without urgent action. Most notably, diabetes deaths are projected to increase by over 80% in upper-middle income countries between 2006 and 2015.

The prevalence of diabetes is rising rapidly especially in the urban population in India. Since 1971 to 2000, a 10 fold increase has been observed (from 1.2% to

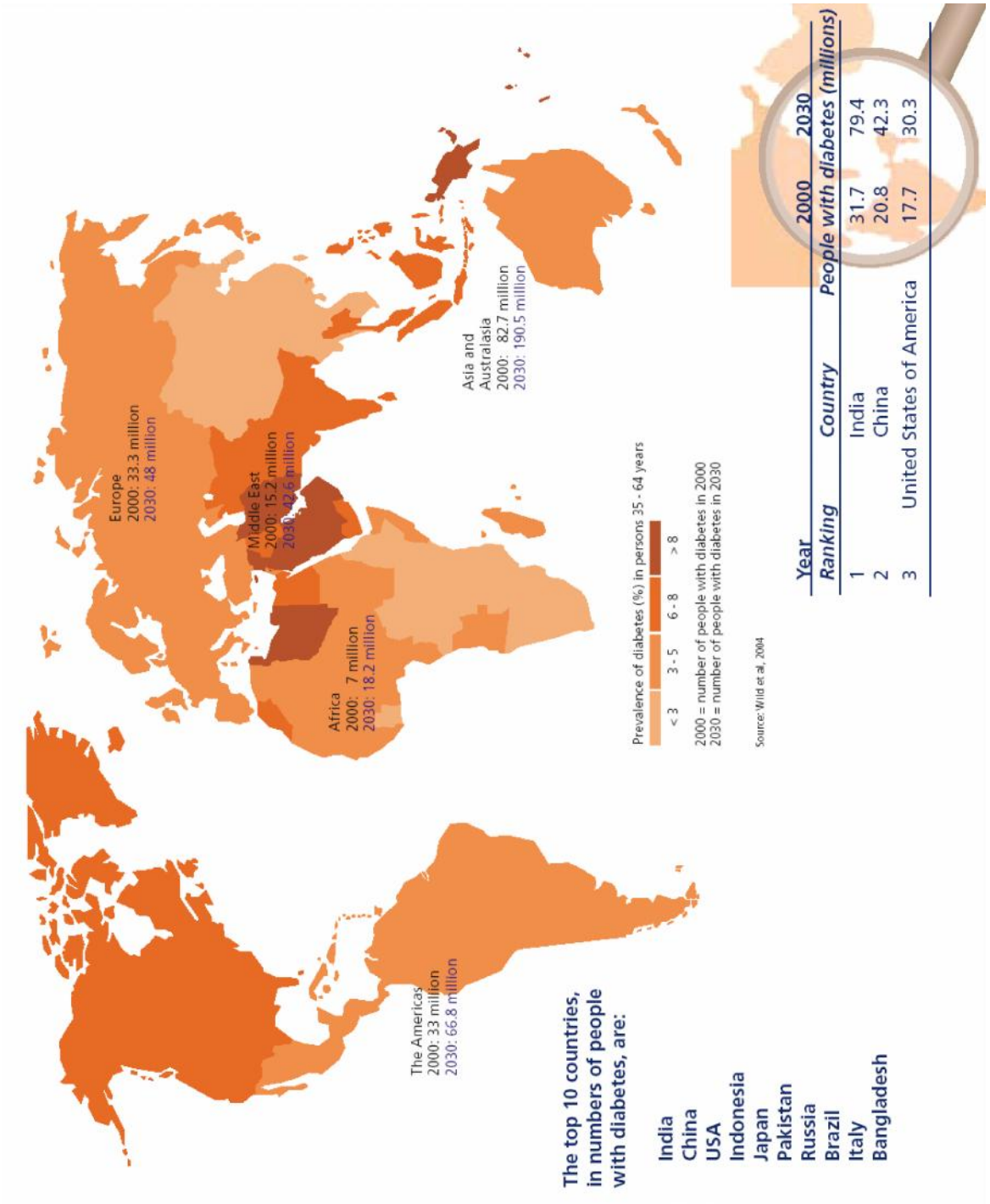
12.1%). It has remained an urban phenomenon so far and all the previous epidemiological studies have illustrated a 4-fold difference in the prevalence of diabetes between the urban and rural population. National urban diabetes survey in 2000 by a group of doctors found that Hyderabad topping the list (16.6% of its population) followed by Chennai (13.5%), Bangalore (12.4%), Kolkatta (11.7%), Delhi (11.6%) and Mumbai (9.3%). The incidence of diabetes in most metros and cities in India presently is 10-15%.[11]

There is a high prevalence of type 2 diabetes mellitus and coronary artery disease among urban and migrant Asian Indians, despite the absence of traditional risk factors. Evidence exists that Asian Indians are more insulin resistant than white persons and that insulin resistance may play an important role in the pathogenesis of these diseases. Increased visceral fat in Asian Indians is associated with increased generalized obesity, which is not apparent from their nano base body mass index. Increased visceral fat is related to dyslipidemia and increased frequency of insulin resistance and may account for the increased prevalence of diabetes mellitus and cardiovascular disease in Asian Indians. In addition, early protein energy deprivation, as indicated by low weight at birth and at 1 year of age, may induce a state of vulnerability to the development of type 2 diabetes in later life, especially if the quantitative and qualitative aspects of nutrition and altered lifestyles during adult years pose an additional challenge[12]. Diabetes is no longer a rich man's disease, the changing life style of Indians has made them prone to type-2 diabetes, earning notorious distinction for India as 'diabetic capital of the world.

Table.1. List of countries with the highest number of estimated cases of diabetes for year 2000 and 2030[13]

Ranking	2000		2030	
	Country	People with diabetes (million)	Country	People with diabetes (million)
1	India	3.7	India	79.4
2	China	20.8	China	42.3
3	US	17.7	US	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russian Federation	4.6	Bangladesh	11.1
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	Bangladesh	3.2	Egypt	6.7

Fig.1. Prevalence of diabetes[14] (Diabetes action now, WHO, 2004)



1.3 Free radicals, diabetes and antioxidant

Much of the morbidity and mortality associated with diabetes is primarily attributed to micro vascular and macro vascular changes, such as atherosclerosis, retinopathy, nephropathy, coronary artery disease, cerebral vascular disease, and peripheral artery disease[15]. One of the reasons for injury related to hyperglycemia is the formation of glycated proteins, glucose oxidation, and increased free fatty acids[16].

Moreover, some recent studies suggest that reactive oxygen species/free radicals may also be involved in the initiation and development of vascular complications in diabetics[17].Oxidative stress combined with mitochondrial dysfunction leads to the activation of inflammatory signaling pathways, which may damage insulin-producing cells and further aggravate the complications of diabetes[18]. Free radicals meet many of the criteria required for a role in the pathogenesis of diabetic vascular disease. They have a direct toxic effect on tissues; and under certain conditions, glucose molecules can also induce free radical production[19]. Free radicals may also modulate oxidative stress in diabetes by nonenzymatic glycosylation of proteins, monosaccharide auto-oxidation, polyol pathway, and indirect production of free radicals through cell damage from other causes[20].

Antioxidants interfere with the production of free radicals and also play a key role to inactivate them. Recent studies[21,22,23] show that majority of the plasma antioxidants (catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GSSG-R), and glutathione peroxidase (GSHPx)) are depleted in Type 2 diabetes patients[24,25,20]. The depletion of antioxidants in the diabetic patients was independent of body mass index and dietary intake and this depletion is a major cause of diabetes-related complications and onset of other disease conditions like atherosclerosis and coronary heart disease[26, 21, 22.] There are numerous reports on perturbed plasma antioxidant levels in patients with diabetes, and most of the studies indicate that supplementation of antioxidants provides greater protection against free radical–induced damage[27,20].It appears therefore, that apart from acting on carbohydrate metabolic targets compounds present in medicinal plants alone or in combination, possess a variety of beneficial activities and have the

potential to impart therapeutic effect holistically in complicated disorders like diabetes and its complications.

1.4 Present status and future prospects

Diabetes is becoming something of a pandemic and despite the recent surge in new drugs to treat and prevent the condition, its prevalence continues to soar. Although several drugs targeted for carbohydrate hydrolysing enzymes (pseudosaccharides), release of insulin from pancreatic b-cells (sulphonyl urea), glucose utilization (biguanides), insulin sensitizers, PPARg agonists (glitazones) are in clinical practice, the growing diabetes market observes a number of changes. The glitazones are meant to target the problem of insulin resistance and enhance insulin action at the cellular level; however, some of these drugs are linked to liver toxicity (troglitazone), including a number of deaths from hepatic failure[28,29,30] and raising the symptoms and risk factors of heart disease leading to heart failure (rosiglitazone) [29]. Therefore, as the long term of risk and effect on the complications of diabetes related with these drugs are not yet clear, UK Drug and Therapeutic Bulletin warrants that patients taking glitazones be monitored for signs of heart failure[31].

On the other hand, traditional medicinal plants with various active principles and properties as discussed in this article have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease and cancer[32,33] . The beneficial multiple activities like manipulating carbohydrate metabolism by various mechanisms, preventing and restoring integrity and function of β-cells, insulin-releasing activity, improving glucose uptake and utilization and the antioxidant properties present in medicinal plants offer exciting opportunity to develop them into novel therapeutics.

The multifactorial pathogenicity of diabetes demands multi-modal therapeutic approach. Thus, future therapeutic strategies require the combination of various types of multiple agents. According to Gale and Lancet that ‘the rise of modern medicine has largely been based on new drugs, and most of us can expect to hobble to our graves on the crutch of polypharmacy’. However, medicatrix naturae –the power of self-preservation or adjustment has been the motto of traditional medicinal practice, which prescribes polyherbal formulations. The

theories of polyherbal formulation have the synergistic, potentiative, agonistic/antagonistic pharmacological agents within themselves due to incorporation of plant medicines with diverse pharmacological actions. These pharmacological principles work together in a dynamic way to produce maximum therapeutic efficacy with minimum side effects. Traditional medicinal preparations therefore, should not be considered just as a collection of therapeutic recipes. They are formulated and prepared keeping in mind the conditions of sickness and the healing properties of individual ingredients. It is important therefore, that herbal medicines and preparations should be taken with the consideration of their holistic therapeutic approach. The multiple activities of plant-based medicinal preparations meant for diabetes offer enormous scope for combating the threat of the diabetic epidemic.

To achieve a blockbuster status, clear evidence of the advantage over the existing therapy is the most important requirement of the day. The ability of modern medicine and health care systems to adequately manage symptoms of chronic and terminal disease is a central theme. The systematic reviews and meta analysis of clinical trials are the foundation of their success. Unfortunately, despite the apparent supremacy in terms of multiple therapeutic approaches of herbal medicines, well-organized, rigorous clinical trail evidences are not adequately available in order to advocate their scientific merit and supremacy over the existing drugs. Though the markets for herbal medicines are booming[34] and evidence for effectiveness is growing, it is also being simultaneously counterbalanced by inadequate regulation[35]**.** Therefore, the product standardization, efficacy, safety and therapeutic risk/benefit associated with the use of herbal medicines need proper evaluation. A sound basic and rigorous clinical investigation to confirm and advocate the excellence over the existing therapies of traditional medicinal plants, preparation(s) mechanism(s) of action and therapeutic effects is absolutely required.

A plenty of traditional herbal medicinary practices have been adopted for the diagnosis, prevention and treatment of various diseases. Many such practices were experimentally proved depicting the scientific insight behind their traditional adoption. These kind of attempts to prove experimentally such traditional practices help in expanding the scope of the usage of herbal drugs.

In the present study, attempt was made to prove the anti-hyperglycemic effect of *Lantana camara.*

Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential[36]. Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques [37, 38,39,40,41]. A wide array of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM [42, 43, 44]. Among these are alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, guani-dine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions. Thus, plants are a potential source of anti-diabetic drugs (and others too) but this fact has not gained enough momentum in the scientific community. The reasons may be many including lack of belief among the practitioners of conventional medicine over alternative medicine, Although, oral hypoglycemic agents/insulin are the mainstay of treatment of diabetes and are effective in controlling hyperglycemia, they have prominent side effects and fail to significantly alter the course of diabetic complications[45]. As the knowledge of heterogeneity of this disorder increases, there is need to look for more efficacious agents with lesser side effects. Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones[46]

The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time. Several medicinal plants have reported to possess potential hypoglycemic activity in Indian system of medicines. There have been several reviews on the hypoglycemic medical plants[47, 48], more particularly use of Indian botanicals for hypoglycemic activity [49, 50, 51].

2. REVIEW OF LITERATURE
DIABETES MELLITUS – A REVIEW

Diabetes

Diabetes Mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins and a risk of complications from vascular disease. It can be classify clinically as either type I diabetes mellitus (type I DM, formally known as insulin dependent diabetes or IDDM) or type 2 disease (type 2 DM, formally known as non-insulin dependent diabetes or NIDDM)[52]. Some facts related to diabetes are given below:

1. Glucose in the blood is produced by the liver from the foods you eat.

2. In a healthy person, the blood glucose level is regulated by several hormones, one of which is insulin. Insulin is produced by the pancreas, a small organ near the stomach that also secretes important enzymes that help in the digestion of food.

3. Insulin allows glucose to move from the blood into liver, muscle, and fat cells, where it is used for fuel.

4. People with diabetes either do not produce enough insulin (type 1 diabetes) or can not use insulin properly (type 2 diabetes), or both.

5. In diabetes, glucose in the blood cannot move into cells, and it stays in the blood. This not only harms the cells that need the glucose for fuel, but also harms certain organs and tissues exposed to the high glucose levels.

2.1 *Pancreas*

The human pancreas is an amazing organ with two main functions: [1] to produce pancreatic endocrine hormones (e.g., insulin & glucagon) which help regulate many aspects of our metabolism and [2], to produce pancreatic digestive enzymes. Pancreatic production of insulin, somatostatin, gastrin, and glucagon plays an important role in maintaining sugar and salt balance in our bodies and therefore any problem in the production or regulation of these hormones will manifest itself with problems with blood sugar and fluid / salt imbalances. The external secretion of the

pancreas is digestive in function, and the endocrine functions are performed by the *islets of Langerhans*. They are small, highly vascularized masses of cells scattered throughout the pancreas, forming only 1 to 3 percent of the entire organ. Pancreatic islets are scattered throughout the pancreas. Like all endocrine glands, they secrete their hormones into the bloodstream and not into tubes or ducts like the digestive pancreas. Because of this need to secrete their hormones into the blood stream, pancreatic islets are surrounded by small blood vessels.

The islets of Langerhans contain four type of secretory cells: *1)* Alpha (A) cell, secretes glucagons *2)* Beta (B) cells, secretes insulin *3)* Delta (D) cells, secretes somatostatin *4)* PP (F) cells, secretes pancreatic polypeptide[53].

2.2 *Insulin*

Insulin is the main hormone controlling intermediary metabolism. It is secreted from the islets of Langerhans and islets of Langerhans present in pancreatic B cells. It's most obvious acute effect is to lower blood glucose. Insulin was the first protein for which an amino acid sequence was determined. It consists of two peptide chains (A and B, of 21 and 30 amino acid residue, respectively). Much of the carbohydrate in food is converted within a few hours to the monosaccharide glucose, the principal carbohydrate in blood. Some carbohydrates are not; fruit sugar (fructose) is usable as cellular fuel but is not converted to glucose and does not participate in the insulin / glucose metabolic regulatory mechanism, nor does the carbohydrate cellulose (though it is actually many glucoses in long chains) as humans and many animals have no digestive pathway capable of handling it. Insulin is released into the blood by beta cells (β-cells) in the pancreas in response to rising levels of blood glucose (e.g., after a meal). Insulin enables most body cells (about 2/3 is the usual estimate, including muscle cells and adipose tissue) to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose (the basic sugar used for fuel) to glycogen for internal storage in liver and muscle cells. Reduced insulin levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall, although only glucose thus recovered by

the liver re-enters the bloodstream as muscle cells lack the necessary export mechanism[54]

2.2.1 *Synthesis and secretion*

Insulin is synthesized as a precursor (preproinsulin) in the rough endoplasmic reticulum. Preproinsulin is transported to the Golgi apparatus where it undergoes proteolytic cleavage first to proinsulin and then to insulin plus a fragment of uncertain function called C-peptide. Insulin and C-peptide are stored in granules in B-cells. The main factor controlling the synthesis and secretion of insulin in the blood glucose concentration. B-cells respond both the absolute glucose concentration and also to the rate of change of blood glucose. There is a steady basal release of insulin and also a response to a change in blood glucose. The response to an increase blood glucose has two phases – an initial rapid phase reflecting release of stored hormone and a slower, delayed phase reflecting both continued release of stored hormone and new synthesis.

ATP-sensitive potassium channels determine the resting membrane potential in B-cells. Glucose enters B-cells via a membrane transporter called Glut-2, and its subsequent metabolism via glucokinase (the rate limiting enzyme that act as the "glucose sensor" linking insulin secretion to extracellular glucose) and glycolysis increases intracellular ATP. This blocks K_{ATP} causing membrane depolarization and opening of voltage-dependent calcium channels, leading to Ca^{2+} influx. This Ca^{2+} signal induces insulin secretion, but only in the presence of amplifying messengers including diacylglycerol (DOC), non-esterified arachidonic acid. Phaspholipases are commonly activated by Ca^{2+}, but free arachidonic acid is liberated in B-cells by an ATP-sensitive Ca^{2+}-insensitive (ASCI) phaspholipase A_2. Consequently, in B-cells, Ca^{2+} entry and arachidonic acid production are both driven by ATP, linking cellular energy status to insulin secretion

2.2.2 Actions of insulin

Insulin is the main hormone controlling intermediary metabolism, having action on liver, muscle and fat. It reduces blood sugar. On the molecular aspects of its mechanism are discussed below.

2.2.3 *Effect of insulin on carbohydrate metabolism*

Insulin influences glucose metabolism in most tissues, especially the liver where it inhibits glycogenolysis (glycogen breakdown) and gluconeogenesis (synthesis of glucose from non-carbohydrate sources) while stimulating glycogen synthesis. It also increase glucose utilization (glycolysis), but overall effect is to increase hepatic glycogen stores. In muscle, unlike liver, uptake of glucose is slow and is the rate-limiting step in carbohydrate metabolism. The main effect of insulin is to increase facilitated transport of glucose via a transport called Glut-4, and to stimulate glycogen synthesis and glycolysis. Insulin increase glucose uptake by Glut-4 in adipose tissue as well as muscle, enhancing glucose metabolism.

2.2.4 *Effect of insulin on fat metabolism*

Insulin increase the fatty acid as well as triglyceride synthesis in adipose tissue and liver. It inhibit lipolysis, partly by dephosphorylation of lipase. It also inhibits the lipolytic actions of adrenaline, growth hormone and glucagons by opposing their action on adenylate cyclase.

2.2.5 *Effect of insulin on protein metabolism*

Insulin stimulates uptake of amino acids into muscle and increase protein catabolism and inhibit oxidation of amino acid in the liver.

2.2.6 *Other metabolic effects of insulin*

Other metabolic effect of insulin include transport into cells of K^+, Ca^{2+}, nucleosides and inorganic phosphate.

2.2.6 Distribution and degradation of insulin

Insulin circulates in the blood as the free monomer, and its volume of distribution approximates the volume of extracellular fluid. Under fasting conditions, the pancreas secretes about 1 U of insulin per hour into the portal vein to achieve a concentration of insulin in the portal blood of 50 to 100 µU/ml. the half life of insulin in plasma is about 5 to 6 minutes in normal and uncomplicated diabetic subjects.[55]

Degradation of insulin occurs primarily in liver, kidney, and muscle. About 50% of the insulin that reaches the liver by the portal vein is destroyed and never reaches general circulation. Insulin is filtered by renal glomeruli and is reabsorbed by the tubules, which also degrade it. Several enzyme involved in degradation of insulin, the primary insulin degradation enzyme is thiol metalloproteinase. It is primarily localized in hepatocytes[56].

2.3 Glucose metabolism

Because insulin is the principal hormone that regulates uptake of glucose into most cells from the blood (primarily muscle and fat cells, but not central nervous system cells), deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

Much of the carbohydrate in food is converted within a few hours to the monosaccharide glucose, the principal carbohydrate found in blood. Some carbohydrates are not converted. Notable examples include fruit sugar (fructose) that is usable as cellular fuel, but it is not converted to glucose and does not participate in the insulin / glucose metabolic regulatory mechanism; additionally, the carbohydrate cellulose (though it is actually many glucose molecules in long chains) is not converted to glucose, as humans and many animals have no digestive pathway capable of handling cellulose. Insulin is released into the blood by beta cells (β-cells) in the pancreas in response to rising levels of blood glucose (e.g., after a meal) (Fig 1). Insulin enables most body cells (about 2/3 is the usual estimate, including muscle cells and adipose tissue) to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Insulin is also the principal control signal for conversion of glucose (the basic sugar used for fuel) to glycogen for internal storage in liver and muscle cells. Reduced glucose levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when glucose levels fall, although only glucose thus recovered by the liver re-enters the bloodstream as muscle cells lack the necessary export mechanism.

Higher insulin levels increase many anabolic ("building up") processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin is the principal

signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa. In particular, it is the trigger for entering or leaving ketosis (ie, the fat burning metabolic phase).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, glucose will not be handled properly by body cells (about ⅔ require it) or stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis.

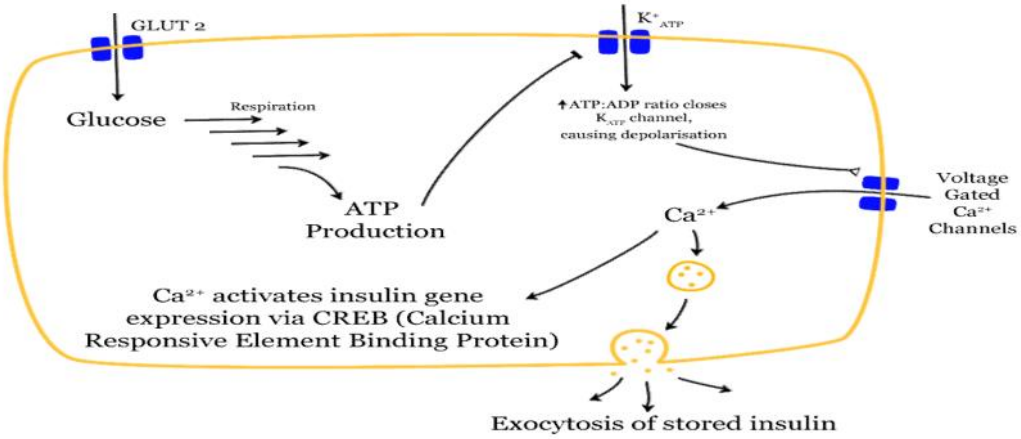


Fig. No.2. Mechanism of insulin release in normal pancreatic beta cells. Insulin production is more or less constant within the beta cells, irrespective of blood glucose levels. It is stored within vacuoles pending release, via exocytosis, which is triggered by increased blood glucose levels.

2.4 Types of Diabetes

2.4.1 Type 1 diabetes mellitus

Formerly known as insulin-dependent diabetes (IDDM), childhood diabetes or also known as juvenile diabetes, is characterized by loss of the insulin-producing beta cells of the islets of Langerhans of the pancreas leading to a deficiency of insulin. It should be noted that there is no known preventative measure that can be taken against type 1 diabetes. Most people affected by type 1 diabetes are otherwise healthy and of a healthy weight when onset occurs. Diet and exercise cannot reverse or prevent type 1 diabetes. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. This type comprises up to 10% of total cases in North America and

Europe, though this varies by geographical location. This type of diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of cases of diabetes affecting children.

The main cause of beta cell loss leading to type 1 diabetes is a T-cell mediated autoimmune attack[57]. The principal treatment of type 1 diabetes, even from the earliest stages, is replacement of insulin. Without insulin, ketosis and diabetic ketoacidosis can develop and coma or death will result.

Currently, type 1 diabetes can be treated only with insulin, with careful monitoring of blood glucose levels using blood testing monitors. Emphasis is also placed on lifestyle adjustments (diet and exercise). Apart from the common subcutaneous injections, it is also possible to deliver insulin by a pump, which allows continuous infusion of insulin 24 hours a day at preset levels and the ability to program doses (a bolus) of insulin as needed at meal times. An inhaled form of insulin, Exubera, was approved by the FDA in January 2006[58].

Type 1 treatment must be continued indefinitely. Treatment does not impair normal activities, if sufficient awareness, appropriate care, and discipline in testing and medication is taken. The average glucose level for the type 1 patient should be as close to normal (80–120 mg/dl, 4–6 mmol/l) as possible. Some physicians suggest up to 140–150 mg/dl (7-7.5 mmol/l) for those having trouble with lower values, such as frequent hypoglycemic events. Values above 200 mg/dl (10 mmol/l) are often accompanied by discomfort and frequent urination leading to dehydration. Values above 300 mg/dl (15 mmol/l) usually require immediate treatment and may lead to ketoacidosis. Low levels of blood glucose, called hypoglycemia, may lead to seizures or episodes of unconsciousness.

2.4.2 Type 2 diabetes mellitus

Previously known as adult-onset diabetes, maturity-onset diabetes, or non-insulin-dependent diabetes mellitus (NIDDM)—is due to a combination of defective insulin secretion and *insulin resistance* or *reduced insulin sensitivity* (defective responsiveness of tissues to insulin), which almost certainly involves the insulin receptor in cell membranes. In the early stage the predominant abnormality is reduced insulin sensitivity, characterized by elevated levels of insulin in the blood. At this stage hyperglycemia can be reversed by a variety of measures and medications that

improve insulin sensitivity or reduce glucose production by the liver, but as the disease progresses the impairment of insulin secretion worsens and therapeutic replacement of insulin often becomes necessary. There are numerous theories as to the exact cause and mechanism for this resistance, but central obesity (fat concentrated around the waist in relation to abdominal organs, and not subcutaneous fat, it seems) is known to predispose individuals for insulin resistance, possibly due to its secretion of adipokines (a group of hormones) that impair glucose tolerance. Abdominal fat is especially active hormonally. Obesity is found in approximately 55% of patients diagnosed with type 2 diabetes (UKPDS, 1998). Other factors include aging (about 20% of elderly patients are diabetic in North America) and family history (type 2 is much more common in those with close relatives who have had it), although in the last decade it has increasingly begun to affect children and adolescents, likely in connection with the greatly increased childhood obesity seen in recent decades in some places.

Type 2 diabetes may go unnoticed for years in a patient before diagnosis, as visible symptoms are typically mild or non-existent, usually without ketoacidotic episodes, and can be sporadic as well. However, severe long-term complications can result from unnoticed type 2 diabetes, including renal failure due to diabetic nephropathy, vascular disease (including coronary artery disease), vision damage due to diabetic retinopathy, loss of sensation or pain due to diabetes neuropathy, liver damage from non-alcoholic steatohepatitis, etc.

Type 2 diabetes is usually first treated by attempts to change physical activity (generally an increase is desired), the diet (generally to decrease carbohydrate intake), and weight loss. These can restore insulin sensitivity, even when the weight loss is modest, for example, around 5 kg (10 to 15 lb), most especially when it is in abdominal fat deposits. Some type 2 diabetics can achieve satisfactory glucose control, sometimes for years, as a result. However, the underlying tendency to insulin resistance is not lost, and so attention to diet, exercise, and weight loss must continue. The usual next step, if necessary, is treatment with oral antidiabetic drugs. As insulin production is initially only moderately impaired in type 2 diabetics, oral medication (often used in various combinations) can still be used to improve insulin production (e.g., sulfonylureas), to regulate inappropriate release of glucose by the liver (and attenuate insulin resistance to some extent (e.g., metformin), and to substantially

attenuate insulin resistance (e.g., thiazolidinediones). According to one study, overweight patients treated with metformin compared with diet alone, had relative risk reductions of 32% for any diabetes endpoint, 42% for diabetes related death and 36% for all cause mortality and stroke (Armenian Medical Network, 2006). When oral medications fail (cessation of beta cell insulin secretion is not uncommon amongst Type 2s), insulin therapy will be necessary to maintain normal or near normal glucose levels. A disciplined regimen of blood glucose checks is recommended, most particularly and necessarily when taking medications.

2.4.3 Type 3 diabetes mellitus (Gestational diabetes)

Gestational diabetes also involves a combination of inadequate insulin secretion and responsiveness, resembling type 2 diabetes in several respects. It develops during pregnancy and may improve or disappear after delivery. Even though it may be transient, gestational diabetes may damage the health of the fetus or mother, and about 20%–50% of women with gestational diabetes develop type 2 diabetes later in life.

Gestational diabetes mellitus (GDM) occurs in about 2%–5% of all pregnancies. It is temporary and fully treatable but, if untreated, may cause problems with the pregnancy, including macrosomia (high birth weight), fetal malformation and congenital heart disease. It requires careful medical supervision during the pregnancy.

Fetal/neonatal risks associated with GDM include congenital anomalies such as cardiac, central nervous system, and skeletal muscle malformations. Increased fetal insulin may inhibit fetal surfactant production and cause respiratory distress syndrome. Hyperbilirubinemia may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental profusion due to vascular impairment. Induction may be indicated with decreased placental function. Cesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

2.4.4 Other types of diabetes

There are several rare causes of diabetes mellitus that do not fit into type 1, type 2, or gestational diabetes:

1. Genetic defects in beta cells (autosomal or mitochondrial): Both type 1 and type 2 diabetes are at least partly inherited. Type 1 diabetes appears to be triggered by some (mainly viral) infections, or in a less common group, by stress or environmental exposure (such as exposure to certain chemicals or drugs). There is a genetic element in individual susceptibility to some of these triggers which has been traced to particular HLA genotypes (i.e., the genetic "self" identifiers relied upon by the immune system). However, even in those who have inherited the susceptibility, type 1 diabetes mellitus seems to require an environmental trigger. A small proportion of people with type 1 diabetes carry a mutated gene that causes maturity onset diabetes of the young (MODY).

   Wolfram's syndrome - Wolfram's syndrome is an autosomal recessive neurodegenerative disorder that first becomes evident in childhood. It consists of diabetes insipidus, diabetes mellitus, optic atrophy, and deafness, hence the acronym DIDMOAD (Armenian Medical Network, 2006).

2. There is a stronger inheritance pattern for type 2 diabetes. Those with first-degree relatives with type 2 have a much higher risk of developing type 2, increasing with the number of those relatives. Concordance among monozygotic twins is close to 100%, and about 25% of those with the disease have a family history of diabetes. Candidate genes include *KCNJ11* (potassium inwardly rectifying channel, subfamily J, member 11), which encodes the islet ATP-sensitive potassium channel Kir6.2, and *TCF7L2* (transcription factor 7–like 2), which regulates proglucagon gene expression and thus the production of glucagon-like peptide-1(Rother, 2007).

   Genetically-related insulin resistance, with or without lipodystrophy (abnormal body fat deposition)

3. Diseases of the pancreas (e.g. chronic pancreatitis, cystic fibrosis)
4. Hormonal defects
5. Chemicals or drugs

The tenth version of the International Statistical Classification of Diseases (ICD-10) contained a diagnostic entity named "malnutrition-related diabetes mellitus" (MRDM or MMDM, ICD-10 code E12). A subsequent WHO 1999 working group recommended that MRDM be deprecated, and proposed a new taxonomy for alternative forms of diabetes[59]. Classifications of non-type 1, non-type 2, non-gestational diabetes remains controversial.

Another risk factor is obesity, particularly central obesity (i.e., that in and around abdominal organs), which is found in approximately 85% of North American patients diagnosed with this type, so some experts believe that inheriting a tendency toward obesity also contributes.

2.5 Diabetes Causes

Type 1 diabetes is believed to be an autoimmune disease. The body's immune system attacks the cells in the pancreas that produce insulin.

- A predisposition to develop type 1 diabetes may run in families but much less so than for type 2.
- Environmental factors, such as certain types of viral infections, may also contribute.
- Type 1 diabetes is most common in people of non-Hispanic white persons of Northern European descent, followed by African Americans and Hispanic Americans. It is relatively rare in those of Asian descent.
- Type 1 diabetes is slightly more common in men than in women.

Type 2 diabetes: Type 2 diabetes is believed to have a strong genetic link, meaning that it tends to run in families. Several genes are being studied that may be related to the cause of type 2 diabetes. Risk factors for developing type 2 diabetes include the following:

- High blood pressure
- High blood triglyceride (fat) levels
- Gestational diabetes or giving birth to a baby weighing more than 9 pounds
- High-fat diet
- High alcohol intake
- Sedentary lifestyle

- Obesity or being overweight
- Ethnicity: Certain groups, such as African Americans, Native Americans, Hispanic Americans, and Japanese Americans, have a greater risk of developing type 2 diabetes than non-Hispanic whites.
- Aging: Increasing age is a significant risk factor for type 2 diabetes. Risk begins to rise significantly at about age 45 years, and rises considerably after age 65 years.

2.6 Symptoms of Diabetes

- Unusual thirst,
- frequent and profuse urination,
- loss of weight despite increased appetite and food intake,
- weakness and drowsiness,
- Itching of the skin and boils.

2.7 Complications of diabetes

Both forms of diabetes ultimately lead to high blood sugar levels, a condition called hyperglycemia. Over a long period of time, hyperglycemia damages the retina of the eye, the kidneys, the nerves, and the blood vessels.

- Damage to the retina from diabetes (diabetic retinopathy) is a leading cause of blindness.
- Damage to the kidneys from diabetes (diabetic nephropathy) is a leading cause of kidney failure.
- Damage to the nerves from diabetes (diabetic neuropathy) is a leading cause of foot wounds and ulcers, which frequently lead to foot and leg amputations.
- Damage to the nerves in the autonomic nervous system can lead to paralysis of the stomach (gastroparesis), chronic diarrhea, and an inability to control heart rate and blood pressure with posture changes.

- Diabetes accelerates atherosclerosis, or the formation of fatty plaques inside the arteries, which can lead to blockages or a clot (thrombus), which can then lead to heart attack, stroke, and decreased circulation in the arms and legs (peripheral vascular disease).

- Diabetes predisposes people to high blood pressure and high cholesterol and triglyceride levels. These independently and together with hyperglycemia increase the risk of heart disease, kidney disease, and other blood vessel complications.

In the short run, diabetes can contribute to a number of acute (short-lived) medical problems.

- Many infections are associated with diabetes, and infections are frequently more dangerous in someone with diabetes because the body's normal ability to fight infections is impaired. To compound the problem, infections may worsen glucose control, which further delays recovery from infection.

- Hypoglycemia, or low blood sugar, occurs from time to time in most people with diabetes. It results from taking too much diabetes medication or insulin (sometimes called insulin reaction), missing a meal, doing more exercise than usual, drinking too much alcohol, or taking certain medications for other conditions. It is very important to recognize hypoglycemia and be prepared to treat it at all times. Headache, feeling dizzy, poor concentration, tremors of hands, and sweating are common symptoms of hypoglycemia. You can faint or have a seizure if blood sugar level gets too low.

- Diabetic ketoacidosis is a serious condition in which uncontrolled hyperglycemia (usually due to complete lack of insulin or a relative deficiency of insulin) over time creates a buildup in the blood of acidic waste products called ketones. High levels of ketones can be very harmful. This typically happens to people with type 1 diabetes who do not have good blood glucose control. Diabetic ketoacidosis can be precipitated by infection, stress, trauma, missing medications like insulin, or medical emergencies like stroke and heart attack.

- Hyperosmolar hyperglycemic nonketotic syndrome is a serious condition in which the blood sugar level gets very high. The body tries to get rid of the excess blood sugar by eliminating it in the urine. This increases the amount of urine significantly and often leads to dehydration so severe that it can cause seizures, coma, even death. This syndrome typically occurs in people with type 2 diabetes who are not controlling their blood sugar levels or have become dehydrated or have stress, injury, stroke, or medications like steroids.

2.8 Medications

Many different types of medications are available to help lower blood sugar levels in type 2 diabetes. Each type works in a different way. It is very common to combine 2 or more types to get the best effect with fewest side effects.

- Sulfonylureas: These drugs stimulate your pancreas to make more insulin.
- Biguanides: These agents decrease the amount of glucose produced by your liver.
- Alpha-glucosidase inhibitors: These agents slow absorption of the starches you eat. This slows down glucose production.
- Thiazolidinediones: These agents increase your sensitivity to insulin.
- Meglitinides: These agents stimulated the pancreas to make more insulin.
- D-phenylalanine derivatives: These agents stimulate your pancreas to produce more insulin more quickly.
- Amylin synthetic derivatives: Amylin is a naturally occurring hormone secreted by the pancreas along with insulin. An amylin derivative, such as pramlintide (Symlin), is indicated when blood sugar control is not achieved despite optimal insulin therapy. Pramlintide is administered as a subcutaneous injection along with insulin and helps achieve lower blood sugar levels after meals, helps reduce fluctuation of blood sugar levels throughout the day, and improves hemoglobin A1C levels.

- Incretin mimetics: Incretin mimetics promote insulin secretion by the pancreas and mimic other blood sugar level lowering actions that naturally occur in the body. Exdenatie (Byetta) is the first incretin mimetic agent approved in the United States. It is indicated for diabetes mellitus type 2 in addition to metformin or a sulfonylurea when these agents have not attained blood sugar level control alone.

- Insulins: Human insulin is the only type of insulin available in the United States; it is less likely to cause allergic reactions than animal-derived varieties of insulin. The type of insulin chosen to customize treatment for an individual is based on the goal of providing optimal blood sugar control. Different types of insulin are available and categorized according to their times of action onset and duration. Commercially prepared mixtures of some insulins may also be used to provide constant (basal) control and immediate control[60].

    - Rapid-acting insulins
        - Regular insulin (Humulin R, Novolin R)
        - Insulin lispro (Humalog)
        - Insulin aspart (Novolog)
        - Insulin glulisine (Apidra)
        - Prompt insulin zinc (Semilente, slightly slower acting)
        - Inhaled insulin (Exubera)
    - Intermediate-acting insulins
        - Isophane insulin, neutral protamine Hagedorn (NPH) (Humulin N, Novolin N)
        - Insulin zinc (Lente)
    - Long-acting insulins
        - Extended insulin zinc insulin (Ultralente)
        - Insulin glargine (Lantus)
        - Insulin detemir (Levemir)

2.9 Free radicals

2.9.1 Chemistry of Free radicals

In every atom, electrons inhabit shells or orbits around the nucleus. Each orbit has sub - orbits known as orbitals. Each orbital can accommodate two electrons and it is stable if it has two electrons. If one electrons in the pair is lost or missing, then the atom or molecule has an intense thirst to retrieve the electron from other molecules to attain stability. Such an unstable and reactive atom (or molecule) is called a free radical. These free radicals are capable of independent existence and have an unpaired electron in an orbital (12, 14). Free radicals are natural by-products of our own metabolism. These are electrically charged molecules that attack our cells, tearing through cellular membranes to react and create havoc with the nucleic acids, proteins, and enzymes present in the body. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. Free radicals may be designated as molecular sharks that damage molecules in cell membranes, in mitochondria (the cells energy plants), and in the DNA (the cell's intelligence) and are very unstable, tend to rob electrons from the molecules in the immediate surrounding in order to replace their own losses.

The energy required to dissociate the covalent bond can be provided by heat, electromagnetic radiation etc. The presence of an unpaired electron increases reactivity as the solitary electron seeks a partner for stability. As partner can be obtained by abstracting an electron from a co-reactant. This reaction results in quenching by reduction (election addition) of the radical and formation of a new radical by oxidation (electron loss) of the co-reactant.

Oxygen is a good example of "friend and foe" as the same molecule is essential for survival and is also toxic beyond a certain level. Oxygen is vital for aerobic life processes. However about 5% or more of the inhaled oxygen is converted to reactive oxygen species (ROS) (11). Though oxygen can behave like a radical (diradical) owing to the presence of 2 unpaired electrons of parallel spin, it does not exhibit extreme reactivity due to quantum mechanical reactions (11). Its electronic structure results in formation of water by reduction with four electrons i.e.

$$O_2 + 4H^+ + 4\,e^- \longrightarrow 2H_2O$$

The toxicity of oxygen to humans has been of interest in relation to diving underwater, swimming and in the use of hyperboric oxygen in the treatment of cancer, infections, multiple sclerosis and lung diseases. High pressure oxygen frequently causes acute central nervous system (CNS) toxicity in animals producing convulsions.

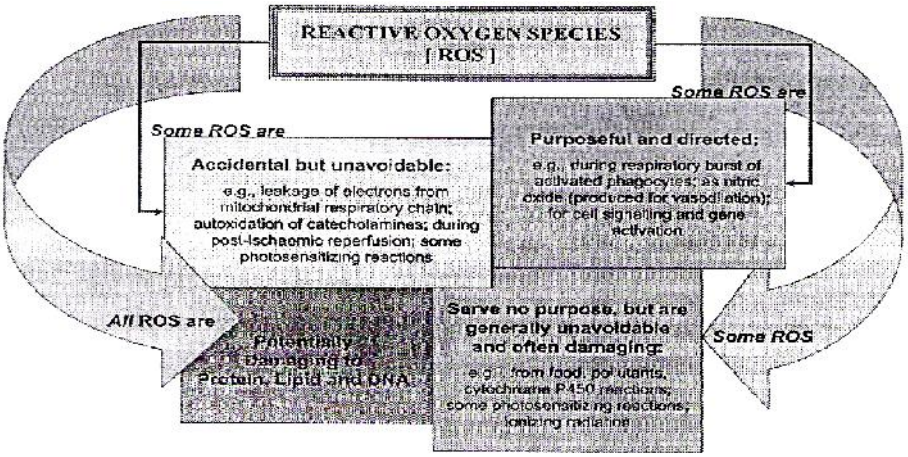
ROS is a collective term, which includes not only the oxygen radicals ($O_2^{\bullet -}$, and $OH^{\bullet}$) but also some non radical derivatives of oxygen. These include H_2O_2, HOC1 and ozone (O_3). ROS.

Radicals	Non radicals
Superoxide radical ($O_2^{\bullet -}$)	H_2O_2
Hydroxyl radical ($OH^{\bullet}$)	O3
Peroxyl radical ($RO^{\bullet}$)	Singlet oxygen
Alkoxyl radical ($RO^{\bullet}$)	Peroxynitrile
Hydroperoxyl radical	(ONOO)
($HO_2^{\bullet}$)	

Various sources of ROS have been identified in living organism. The superoxide anion radical appears to play the central role and other reactive intermediates are formed from it. ROS are also produced in the organism as a part of the primary immune defense. Phagocytic cells such as neutrophils or marophages defend against foreign organisms by generating $O_2^{\bullet -}$ and nitric oxide as a part of the killing mechanism. These ROS can be studied under the two headings biologically important radicals and biologically important non-radicals.

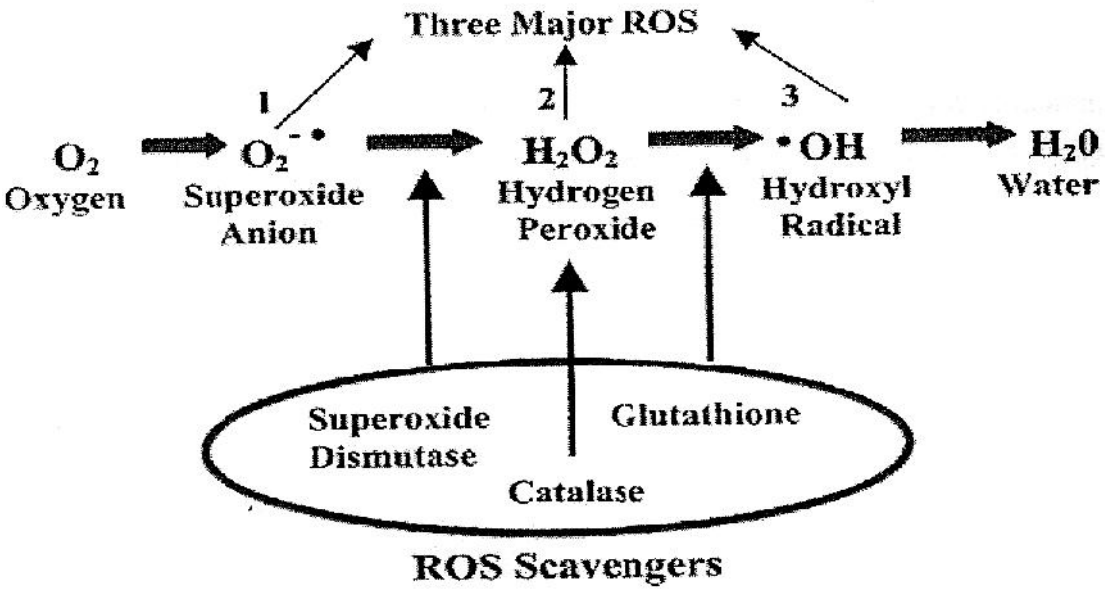
1. High impact energy sources (thermal, microwave etc)
2. Metals (Cadmium, copper, iron, mercury, Zinc etc)
3. Enzymes (metallic or otherwise) dispersed or grouped in cytoplasmic organelles
4. Mechanic action
5. Toxic agents (alcohol, pesticides, cigarettes, air pollutants etc)
6. Physical and psychological stress

7. Sources and characteristics of ROS



Any free radical involving oxygen is then referred to as reactive exygen species (ROS). The most commonly formed ROS are superoxide anion radcial ($O_2^{\bullet}$-) and hydroxyl radical (**.**OH). $O_2^{\bullet}$ - is formed when one electron is added to an oxygen molecule, and is considered the least reactive type of ROS. Once $O_2^{\bullet}$ - is produced, it triggers a rapid cascade of events that creates other free radicals, eventually terminating in the formation of H_2O. In humans, $O_2^{\bullet}$-is the most commonly produced free radical. Phagocytic cells such as macrophage and neutrophils are prominent sourced of $O_2^{\bullet}$-. In an inflammatory response, these cells generate free radicals that attack invading pathogens such as bacteria. Production of $O_2^{\bullet}$ - by activated phagocytic cells in response to inflammation is one of the most studied free radical-producing systems[61] . If oxygen attracts two hydrogen molecules, hydrogen peroxide (H_2O_2) is formed. H_2O_2, though not technically considered an oxygen free radical, is a member of the ROS family and may selectively participate in free radical generation. The majority of the H_2O_2 is broken down to oxygen and water by the cellular enzyme catalase. In addition to catalase, the enzyme gluatione perioxidase is responsible for the break down of H_2O_2 and any peroxides that form on lipids within the body. The hydroxyl radical ($^{\cdot}$OH) is the most reactive of the free radical molecules. The hydroxyl radical damages cell membranes and lipoproteins by a process called lipid peroxidation. Lipid peroxidation damage to lipids in low density lipoprotein (LDL)

plays an important role in atherosclerosis. The process of formation of reactive oxygen species (ROS) shown below.



2.9.2Biologically important radicals

Nitric oxide, sulphur radicals, peroxyl and alkoxyl radicals, superoxide radical, Hydroxyl radical, Transition metals.

2.9.3Free radicals and diseases

Despite the existence of endogenous defense mechanisms against ROS, it has been observed that whenever either the level of the cellular antioxidant system goes down or when the ROS reach abnormally high levels, oxidative damage to the cells occurs leading to several pathological conditions. Over about 100 disorders like rheumatoid arthritis (RA), hemorrhagic shock, CVS disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases, gastrointestinal ulcerogenesis and AIDS have been reported as ROS mediators. Some specific examples of ROS mediated diseases include Alzheimer's disease, Parkinson's disease, Atherosclerosis, Cancer, Down's syndrome and ischemic reperfusion injury in different tissues including heart, liver, brain, kidney and GIT. The role played by ROS in stress induced gastric ulcer and inflammatory bowel diseases have been well established, as well as their involvement in the process of ageing.

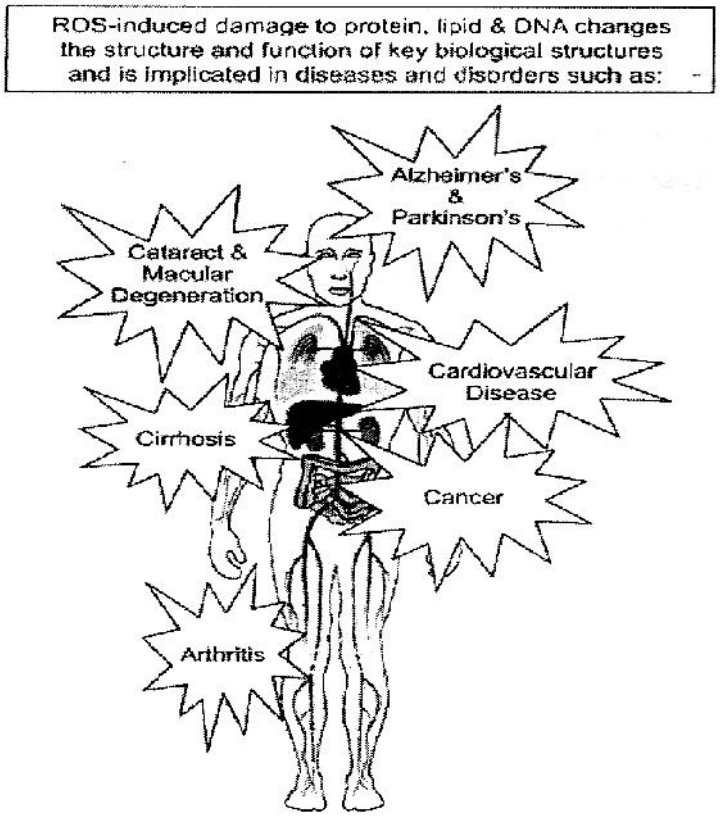


Fig no: 3 ROS in diseases (Source: Comparative Biochemistry and Physiology (11)

In principle, disease associated oxidative stress could result from either or both of the following.

1. Diminished antioxidants due to the mutations affecting antioxidant enzymes like SOD, GPX or disease that cause depletion of such defenses. Depletion of dietary antioxidants and other essential dietary constituents can also lead to oxidative stress.
2. Increased production of ROS/RNS by exposure to elevated oxygen, the presence of toxins, are metabolized to generate ROS/RNS or excess activation of ROS/RNS producing systems.

It is possible that some cancers may originate as a result of faulty repair following damage produced by free radicals and that the vessel weakness in retrolental fibroplasias is due to LPO, since it can be ameliorated by vitamin E. The hemolysis formerly seen in some premature babies is now rare, probably because of the fortification of infant formulas with vitamin E, but the condition may be related to

LPO. In most diseases however, increased oxidant formation is a consequence of the disease activity. For example, infiltration of a large number of neutrophils into a localized site, followed by activation of these cells to generate ($O_2^{\cdot -}$) and H_2O_2 can produce an intense localized oxidative stress which appears to happen in rheumatoid arthritis and also in some forms of the adult respiratory distress syndrome.

Atherosclerosis, Hypertension, Diabetes are perfusion injury, Inflammation, Autoimmune diseases, Rheumatoid arthritis, Asthma, Cystic fibrosis, cancer, Parkinson's disease (PD), Free radicals in aging biology.

2.9.4 Antioxidant and diabetes

The elevated levels of blood glucose in diabetes produce oxygen free radicals which cause membrane damage due to peroxidation of membrane lipids and protein glycation[62] .Glucose auto-oxidase in the presence of transition metal ions generates oxygen free radicals which make the membrane vulnerable to oxidative damage. The lipid oxidation of the cell membrane has been associated with a number of pathological phenomenon such as increase the membrane rigidity, decrease cellular deformability and lipid fluidity in erythrocytes. The action of diabetes induction agents produced reactive free radicals, which have shown to be cytotoxic to the β-cells of the pancreas[63]. As the diabetogenic action can be prevented by the SOD, CAT, and other hydroxyl radical scavengers such as ethanol, dimethyl urea, there is evidence to suggest that the incidence of diabetes involves superoxide anions and hydroxyl radicals. The harmful effects of superoxide and hydroxyl radicals can be counteracted by antioxidant enzymes such as SOD, CAT, and glutathione peoxidase (GSH-px). In addition to these enzymes, GSH-R and glutathione-S-transferase (GST) provide GSH and help to neutralize toxic electrophiles respectively. There is clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals[64].

Diabetes patient are often stated to be under an oxidative stress. Indeed, the link between diabetes and oxidative stress has been extensively discussed for years, but rigorous experiments to elucidate its importance are still awaited[65]**.** Some diabetogenic agents appear to act by imposing sever oxidative stress on the β-cell. The plasma α-tocopherol levels in diabetic patient are sub-normal, but general

agreement that vitamin C levels are lower than normal in plasma, despite the fact that elevated blood glucose has been reported to inhibit the uptake of ascorbate and of dehydro ascorbate in to calls.

Possible sources of oxidative stress in diabetes include shifts in redox balance resulting from altered carbohydrate and lipid metabolism, increase generation of ROS and decrease levels of antioxidant defenses such as GSH. In physiologic concentrations, endogenous reactive oxygen species (ROS) help to maintain homeostasis. However, when ROS accumulate in excess for prolonged periods of time, they cause chronic oxidative stress and adverse effects. This is particularly relevant and dangerous for islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses.

2.9.5 Antioxidant defence

It has been postulated that the etiology of the complications of diabetes involves oxidative stress perhaps as a result of hyperglycemia[65]. The elevated levels of blood glucose in diabetes produce oxygen-free radicals (OFR), which cause membrane damage due to peroxidation of membrane lipids and protein glycation[66]. Baynes[67] reported that plasma thiobarbituric acid reactive substance (TBARS) levels increased in diabetic patients due to vascular lesions induced by hyperglycemia. Diabetic patients thus have an increased incidence of vascular diseases and it has been suggested that free radical activity increased in diabetes[68]. It has also been shown that glucose under physiological conditions produces oxidants that possess reactivity similar to the hydroxyl-free radicals.

Recent years have witnessed a renewed interest in plants as pharmaceuticals because they synthesize a variety of secondary metabolites with antioxidant potential or antioxidant defence which can play a major role in protection against molecular damage induced by reactive oxygen species (ROS) [69, 70, 71]. The antioxidant defence system represents a complex network with interactions, synergy and specific tasks for a given antioxidant[23]. Recent studies[21, 22, 23] show that majority of the plasma antioxidants are depleted in Type 2 diabetes patients. The depletion of antioxidants in the diabetic patients was independent of body mass index and dietary intake and this depletion is a major cause of diabetes-related complications and onset of other disease conditions[26, 21, and 22]. Antioxidant activities of the majority of compounds have been

reviewed recently[72, 73, 32] and their benefits in oxidative stress and related disease conditions have been widely described. It appears therefore, that apart from acting on carbohydrate metabolic targets compounds present in medicinal plants alone or in combination, possess a variety of beneficial activities and have the potential to impart therapeutic effect holistically in complicated disorders like diabetes and its complications[6].

Table.2. LIST OF PLANTS REPORTED FOR ANTI-DIABETIC ACTIVITY

S.No.	Plant Name	Family
1.	Acacia arabica (Lam.) Muhl. ex Willd.	Mimosaceae
2.	Aegle marmelos (L.) Correa ex Roxb.	Rutaceae
3.	Allium cepa L.	Liliaceae
4.	Allium sativum L.	Alliaceae
5.	Aloe vera (L.) Burm.f.	Aloaceae
6.	Areca catechu L.	Arecaceae
7.	Artemisia pallens Wall. ex DC.	Compositae
8.	Annona squamosa L.	Annonaceae
9.	Andrographis paniculata Nees	Acanthaceae
10.	Aerva lanata (L.) Juss. ex Schult.	Amaranthaceae
11.	Azadirachta indica A. Juss.	Meliaceae
12.	Beta vulgaris L.	Chenopodiaceae
13.	Boerhavia diffusa L.	Nyctaginaceae
14.	Cassia auriculata L.	Leguminosae
15.	Citrullus colocynthis (L.) Schrad.	Cucurbitaceae
16.	Catharanthus roseus (L.) G. Don.	Apocynaceae
17.	Camellia sinensis Kuntze	Theaceae
18.	Eugenia uniflora L.	Myrtaceae
19.	Eucalyptus globulus Labill.	Myrtaceae
20.	Eugenia jambolana Lam.	Myrtaceae
21.	Ficus bengalensis L.	Moraceae

22.	Gymnema montanum Hook.f.	Asclepiadaceae
23.	Gymnema sylvestre R. Br.	Asclepiadaceae
24.	Hibiscus rosa sinensis L.	Malvaceae
25.	Ipomoea batatas (L.) Lam.	Convolvulaceae
26.	Momordica cymbalaria Fenzl ex Naudin	Cucurbitaceae
27.	Momordica charantia L.	Cucurbitaceae
28.	Murraya koeingii (L.) Spreng.	Rutaceae
29.	Ocimum sanctum L.	Lamiaceae
30.	Picrorrhiza kurroa Royle ex Benth.	Scrophulariaceae
31.	Phyllanthus amarus Schumach. & Thonn.	Euphorbiaceae
32.	Pterocarpus santalinus L. f.	Leguminosae
33.	Salacia reticulata Wight.	Celastaceae
34.	Salacia oblonga Wall.	Celastaceae
35.	Swertia chirayita (Roxb. ex Fleming) H. Karst.	Gentianaceae
36.	Terminalia catappa L.	Combretaceae
37.	Terminalia pallida Brandis	Combretaceae
38.	Tinospora cordifolia (Willd.) Hook.f. & Thomson	Menispermaceae
39.	Zingiber officinale Roscoe	Zingiberaceae
40.	Zizyphus sativa Gaertn.	Rhamnaceae

3. PLANT REVIEW
LANTANA CAMARA - A REVIEW

3.1 Plant monograph[74] *Lantana camara*. (Family: Verbenaceae)

3.1.1 Scientific classification

Kingdom	:	Plantae
Order	:	Lamiales
Family	:	Verbenacea
Genus	:	*Lantana*
Species	:	*camara*

3.1.2 Common Names:

Tamil	:	Unnichedi,Arasimala
Hindi	:	Raimuniya
Kannada	:	Kakke
Telegu	:	Pulikampa
Malayalam	:	Kongini, Konkini, Aripoo

3.1.3 Description:

Lantana camara is a thorny shrub upright, half climbing or sometimes more or less hanging, reaching 2-3 m in height. The stems and branches are angular, bearing curved spines, arranged along the edges. The leaves are simple, opposite, decussate with rough lamina, oval, regularly dentate with acute apex. The fruits are small drupes fleshy, about 3 mm in diameter, varying in color from blue to black. Flowers are having a yellow throat, in axillary head almost throughout the year. Stamen four in two pairs, included and ovary two celled, two ovuled. Inflorescences are produced in pairs in the axils of opposite leaves. Inflorescences are compact, dome shaped 2-3 cm across and contain 20-40 sessile flowers. Root system is very strong and it gives out new fresh shoots even after repeated cuttings.

3.1.4 Distribution[75]:

Lantana camara is a tropical origin plant and native to Central and Northern South America and Caribbean. *Lantana camara* is now spreaded to nearly 60 countries viz, New Zealand, Mexico, Florida, Trinidad, Jamaica and Brazil. It is reported in many African countries including Kenya, Uganda, Tanzania and South Africa.

3.1.5 Traditional uses[74]:

Lantana camara has been used in many parts of the world to treat a wide variety of disorders (Ross, 1999).In Central and South America, the leaves were made into a poultice to treat sores, chicken pox and measles. Fevers, colds, rheumatism, asthma and high blood pressure were treated with preparations from the plant (Irvine, 1961). In Ghana, an infusion of the whole plant was used for bronchitis and the powdered root in milk was given to children for stomach-ache (Irvine, 1961). In Asian countries, leaves were used to treat cuts, rheumatism, ulcers and intestinal worms. It has been claimed that a steroid, lancamarone, from the leaves, exhibited cardio tonic properties (Sharma & Kaul,1959) and that lantamine, an alkaloid from the stem, bark and roots showed antipyretic and antispasmodic properties comparable to those of quinine (Sastri,1962).In India the leaves of the plant are boiled for tea and the decoction is a remedy against cough and it is used as a lotion for wounds and Pounded leaves are applied to cuts, ulcers and swellings (Verma RK,2006).

Fig no 4: *Lantana camara* Leaves

3.2 PHARMACOLOGICAL REVIEW

ALLELOPATHIC IMPACT OF *LANTANA CAMARA* ON VEGETATIVE GROWTH AND YIELD COMPONENTS OF GREEN GRAM (*PHASEOLUS RADIATUS*)

K.C. Lenka et.al., (2014) was carried out by An experiment was conducted to understand the allelopathic effects of different concentrations derived from leaf-litter dust of *Lantana camara* on the vegetative growth parameters such as - development of total number of leaves per plant, height of the plant, total leaf area, leaf area index and components of yield such as - production of number of heads per plant, production of seeds per head, weight of seeds, seed yield per plant of green gram (*Phaseolus radiatus*).Results showed different concentrations of leaf-litter dust caused significant inhibitory effect on vegetative growth and yield of the test crop. The study indicates that the allelochemicals released from the leaf-litter dust into the soil suppressed the above parameters of the of the green gram plant.

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF TOPICAL PREPARATION OF *LANTANA CAMARA* LEAVES

K.Lakshman et.al., was carried out by The present study was undertaken to evaluate the analgesic and anti-inflammatory activity of topical preparation of *Lantana camara* leaves, in literature it is found that leaves of *Lantana camara* having analgesic and anti-inflammatory activity. Due to the toxicity of *Lantana camara* on oral administration, topical formulations containing 1%, 2%, 4%, 6% and 8% of alcoholic extract of *Lantana camara* (AELC) were prepared and screened for analgesic and anti-inflammatory activity. Analgesic activity was evaluated with formalin induced paw licking test in mice using Methyl salicylate as a standard. Anti-inflammatory activity was evaluated with carrageenan induced rat paw edema model in wistar rats using Piroxicam gel as a standard. In preliminary phytochemical investigations the AELC was found to contain alkaloids, carbohydrates, glycosides, saponins, proteins, flavonoids, tannins and

phenolic compounds and the pharmacological studies have revealed that preparations containing 4%, 6% and 8% AELC showed significant analgesic and anti-inflammatory activity. In conclusion the formulations containing 4%, 6% and 8% AELC was found to possess good analgesic and anti-inflammatory activity.

ANTIBACTERIAL ACTIVITY OF *LANTANA CAMARA* LINN AND *LANTANA MONTEVIDENSIS* BRIG EXTRACTS FROM CARIRI-CEARÁ, BRAZIL

A review of work was done by **F.S. Baretto et.al., 2010 Jan-Mar; 2(1): 42–44**. The use of medicinal plants with therapeutics properties represents a secular tradition in different cultures, mainly in underdeveloped countries. *Lantana camara* Linn and *Lantana montevidensis* Briq (Verbenaceae) found in tropical and subtropical areas around the world are popularly known as “camará” or “chumbinho.” In popular medicines, both plants are used as antipyretic and carminative and in the treatment of respiratory system infections. In this study, the antibacterial activity of the ethanolic extracts of *L. camara* and *L.montevidensis* leaves and roots against gram-positive and gram-negative strains standard and multi-resistant bacteria isolated from clinical material are presented. In order to determine the minimal inhibitory concentration (MIC), the microdilution method was used. The extracts demonstrated antibacterial activity against all tested bacteria, but the *L. montevidensis* fresh leaves extract present the best result against *P. aeruginosa* (MIC 8 µg/mL) and against multi-resistant *E. coli* (Ec 27) (MIC 16 µg/mL). These results drive new researches with both species in order to isolate the constituents responsible for the activity.

ANTI-LEUKAEMIC ACTIVITY OF *LANTANA CAMARA*

Herbert J.M. *et al.* 1991 was carried by the work Anticancer effect of *Lantana camara*'s root and leaf extracts against Jurkat leukemia cell line was investigated by MTT assay. These extracts had statistically similar antineoplastic property (root IC50, 328.36 ± 53.08 µg/ml; leaf, 394.41 ± 99.73 µg/ml; $p > 0.1$, $n = 3$), averagely 1/10 times as activity as carboplatin (IC50 34.83 ± 3.60 µg/ml; $p < 0.05$, $n = 3$). Decreasing cytotoxicity at higher concentrations implied the existence of

cytoprotective compounds. Morphological examinations indicated apoptosis induction as the mechanism of activity on Jurkat cells. In conclusion, *L. camara*'s root and leaf extracts might be subjects for further fractionation and identification to find new anticancer agents.

ANTIDIABETIC ACTIVITY OF *LANTANA CAMARA* LINN FRUITS IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

Venkatachalam, T et.al., Journal of Pharmacy Research;May2011, Vol. 4 Issue 5, p1550 To evaluate the hypoglycemic activity of methanolic extract of *Lantana camara* linn fruits in normal and streptozotocin induced diabetic rats. Material and methods: Methanolic extract of *Lantana camara* linn fruits were orally tested at the dose of 100 and 200 mg/kg for hypoglycemic activity for normal and streptozotocin induced diabetic rats. In addition changing in body weight, HbA1c, assessed in the methanol-treated diabetic rats, were compared with diabetic control and normal animals. Histopathological observations during 21 days treatment were also evaluated. Statistical analysis: Analysis of Variance (ANOVA) followed by Multiple comparison student two-tail't' test was used for statistical analysis of collected data. Differences were considered significant at $p<0.05$. All the values were indicated in the tables and figures as Mean Â± SEM. Results and discussion: Methanolic extract of *Lantana camara* linn fruit 200 mg/kg produced a significant reduction in fasting blood glucose level in the normal and streptozotocin induced diabetic rats. Significant differences were observed change of body, HbA1c by methanol extract treated- diabetic rats, when compared with the diabetic control and normal animals. Concurrent histopathological studies of the liver these animals showed comparable regeneration by extract which were earlier encored by Streptozotocin.

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF *LANTANA CAMARA* L. EXTRACT IN MICE

T.S.C. SILVA et.al. was carried out by the work of *Lantana camara* L. belongs to the family Verbenaceae, which contains several active compounds in leaves and roots and which are reported to have medicinal and insecticidal properties. Studies

of plants within the same family show the existence of anti-inflammatory activity in paw edema induced by carrageenan, serotonin and histamine and analgesic activity in the acetic acid writhing and tail-flick tests. The present study investigated whether the *L. camara* extract (ACE) also exerts these effects. The ACE toxicity was studied in male mice, and the percentage of mortality recorded 7 days after treatment was assessed. The ACE was evaluated as an antinociceptive agent in the hot plate, tail-flick and acetic acid writhing tests at a nontoxic dose of 1.0 g/Kg. The results showed that 1.5 g/Kg of ACE was not able to cause death, and doses of 3.0 and 4.0 g/Kg caused 50% and 60% death, respectively, in male mice. In all of the antinociceptive tests, 1 g/Kg of ACE markedly reduced responses to pain. Our findings suggest that ACE may have active anti-inflammatory and antinociceptive properties in much smaller doses than toxic.

ANTIULCEROGENIC ACTIVITY OF *LANTANA CAMARA* LEAVES ON GASTRIC AND DUODENAL ULCERS IN EXPERIMENTAL RATS

Bhushan Vyawahare[a], et.al., Journal of Ethnopharmacology Volume 134, Issue 1, 8 March 2011, Pages 195–197 was carried out by A Review was carried out by *Lantana camara* L. (Verbenaceae), a widely growing shrub has been used in the traditional medicine for treating many ailments. The objective of the present study was to evaluate the effects of methanolic extract of *Lantana camara* leaves on gastric and duodenal ulcers. The antiulcerogenic effect of methanolic extract of *Lantana camara* was evaluated in aspirin induced gastric ulcerogenesis in pyloric ligated rats, ethanol induced gastric ulcer, and cysteamine induced duodenal ulcer models. The extract was administered orally at two different doses of 250 mg/kg and 500 mg/kg. The lipid peroxidation, reduced glutathione levels of ethanol induced gastric ulcer model and inhibition zone in diameter against Helicobacter pylori also determined. The *Lantana camara* extract significantly ($P<0.01$) reduced ulcer index, total acidity and significantly ($P<0.01$) increased the gastric pH of aspirin + pylorus-ligation induced ulcerogenesis and ethanol induced gastric ulcer models. The extract also significantly ($P<0.01$) reduced the ulcer index of cysteamine induced duodenal ulcer. The L. camara showed significant ($P<0.01$) reduction in lipid peroxidation and increase in reduced glutathione levels. The inhibition zone in diameter of extract against H. pylori was 20mm. The

methanolic extract of *Lantana camara* leaves shown healing of gastric ulcers and also prevents development of duodenal ulcers in rats.

ANTIULCEROGENIC ACTIVITY OF *LANTANA CAMARA* LEAVES ON GASTRIC AND DUODENAL ULCERS IN EXPERIMENTAL RATS

R. Sathish et.al., Journal of ethnopharmacology 134(1):195-7, 2010 *Lantana camara* L. (Verbenaceae), a widely growing shrub has been used in the traditional medicine for treating many ailments. The objective of the present study was to evaluate the The antiulcerogenic effect of methanolic extract of *Lantana camara* was evaluated in aspirin induced gastric ulcerogenesis in pyloric ligated rats, ethanol induced gastric ulcer, and cysteamine induced duodenal ulcer models. The extract was administered orally at two different doses of 250 mg/kg and 500 mg/kg. The lipid peroxidation, reduced glutathione levels of ethanol induced gastric ulcer model and inhibition zone in diameter against *Helicobacter pylori* also determined.

EVALUATION OF ANTIMOTILITY EFFECT OF LANTANA CAMARA L. VAR. ACUELATA CONSTITUENTS ON NEOSTIGMINE INDUCED GASTROINTESTINAL TRANSIT IN MICE

A review of work was done by **Sagar,et.al., BMC Complementary & Alternative Medicine;2005, Vol. 5, p1** *Lantana camara* L. (Verbenaceae), a widely growing shrub which is toxic to some animal species, has been used in the traditional medicine for treating many ailments. The purpose of the present study was to evaluate the antimotility effects of *Lantana camara* leaf constituents in mice intestine. Methods: Evaluation of antimotility activity was done in intestine of mice treated with *Lantana camara* leaf powder, *Lantana camara* methanolic extract (LCME), lantadene A, neostigmine and neostigmine + LCME. Neostigmine was used as a promotility agent. Intestinal motility was assessed by charcoal meal test and gastrointestinal transit rate was expressed as the percentage of the distance traversed by the charcoal divided by the total length of the small

intestine. The antidiarrheal effect of LCME was studied against castor oil induced diarrhea model in mice. Results: The intestinal transit with LCME at a dose of 500 mg/kg was 26.46% whereas the higher dose (1 g/kg) completely inhibited the transit of charcoal in normal mice. The % intestinal transit in the neostigmine pretreated groups was 24 and 11 at the same doses respectively. When the plant extracts at 125 and 250 mg/kg doses were administered intraperitonealy, there was significant reduction in fecal output compared with castor oil treated mice. At higher doses (500 and 1000 mg/kg), the fecal output was almost completely stopped. Conclusion: The remarkable antimotility effect of *Lantana camara* methanolic extract against neostigmine as promotility agent points towards an anticholinergic effect due to *Lantana camara* constituents and attests to its possible utility in secretory and functional diarrheas and other gastrointestinal disorders. This effect was further confirmed by significant inhibition of castor oil induced diarrhea in mice by various doses of LCME.

STUDIES ON HYPOGLYCAEMIC AND WOUND HEALING ACTIVITIES OF *LANTANA CAMARA* LINN.

A review of work was carried out **by S. Ganapaty et.al.,**To study the antihyperglycaemic and wound healing activity of the leaf extracts of *Lantana camara* Linn. on rats Antihyperglycaemic activity of the aqueous extract of the leaves was evaluated by using both normoglycaemic and alloxan induced hyperglycaemic rats. The wound healing activity was assessed for both leaf juice and hydroalcoholic (ethanol 50% v/v) extract of the leaves on excised rats. The aqueous extract was found to produce significant reduction of blood glucose concentration between 2-4 h of administration in alloxan induced hyperglycaemic rats at tested dose levels. However, in normoglycaemic animals, the extract at 400mg/kg produced significant reduction of blood glucose between 2-4 h of administration. In the wound healing studies, the leaf juice was found to be more active than the extract. In the light of the alarming toxicity of the plant, the use of this plant in whole or any part there of need to be carefully regulated until the toxic principles of the plant are identified and removed, to ensure a safe and effective treatment for diabetes.

NEMATICIDAL ACTIVITY OF *LANTANA CAMARA* L. FOR CONTROL OF ROOT-KNOT NEMATODES

A reviw of work was done by **Ganesh Ghimire et.al.,**Various concentrations of aqueous leaf extract of *Lantana camara* L. were assessed against second stage juveniles (J2) of *Meloidogyne* spp. (Goeldi, 1982) for its nematicidal potency *in vitro* conditions. Study showed 50% concentration of *Lantana camara* leaf extract at 48 hrs of incubation period and above showed effective in immobilizing second stage of larvae (J2) of *Meloidogyne* spp. The standard concentration 'S' (100%) of leaf extract was found to be highly nematostatic, 98.66% of nematode were found dead in 48 hrs. Similarly, 57.66% of nematode juveniles were found dead when applied 50% concentration in 48 hrs. Mean number of (J2) dead at 100% concentration for three time period was statistically significant highest at 48 hrs. So far, 50% concentration in 48 hrs and above was appropriate for controlling the root-knot nematode which seems as an alternative to chemical pesticides.

REPELLENT ACTIVITY OF ESSENTIAL OIL AND LEAF EXTRACT OF *LANTANA CAMARA* L. IN LABORATORY CONDITION

Dr. Sunita Bhargava et.al., was carried out by International Journal of Theoretical & Applied Sciences **5**(1): 170-174(2013) Insect transmitted diseases in tropical countries remain a major health threat causing great morbidity every year. Mosquitoes are animals most of us would rather do without. Several mosquito species belonging to genera *Anopheles, Culex* and *Aedes* are vectors for the pathogens of various diseases like malaria, filariasis, Japanese encephalitis (JE), dengue and dengue haemorrhagic fever, yellow fever, etc. the need to protect ourselves from their bites seems even more important. Many plant-based products are widely used for their insecticidal/repellent properties for control of mosquitoes/protection from mosquito bites. Herbal products with proven potential as insecticide or repellent can play an important role in the interruption of the transmission of mosquito-borne diseases at the individual as well as at the community level. Use of repellents seems to be most reliable method of personal protection against annoyance and infections associated with haematophagous

insects. We have tested the repellency of essential oil extracted from *Lantana camara* in laboratory condition application of oil to the upper surface of the human forearms at the rates between 0.08 to 3.33 mg / cm2 of skin. The study provides evidence for the potential of these essential oil in developing new repellents against mosquitoes.

EVALUATION OF WOUND HEALING ACTIVITY OF ETHANOLIC EXTRACT OF *LANTANA CAMARA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS

A review of work was done by **SIVANAGESWARARAO et.al.,** *Lantana camara* (Verbanacea) is a commonly available medicinal plant throughout India. Wound healing property of the plant in various wound models has been studied. Thorough literature survey revealed that the wound healing property of *Lantana camara* in diabetic wound was not studied. This study was aimed to evaluate the wound healing property of *Lantana camara* in diabetic rats.

4. AIM AND OBJECTIVES

Diabetes Mellitus is considered as one of the five leading causes of death in the world. An epidemic by all standards, nearly 250 million people suffer from diabetes across the world. Synthetic anti-diabetic drugs increase the insulin secretion or decrease the blood glucose level but they are also producing many harmful effects. Since, increase in the use of these drugs in diabetes therapy leads to many side effects and undesirable hazards, there is worldwide trend to go back to natural resources, i.e., traditional plant. In industrialized countries the people are seeking for safer alternative to allopathic medicine because of the increasing realization on the adverse side effects of many modern remedies. The current interest in and demand for herbs is a worldwide phenomenon, WHO currently encourages, recommends and promotes traditional/ herbal remedies in national healthcare programmes because such drugs are easily available at low cost, are comparatively safe and people have faith in such remedies

On the other hand, the therapeutic approach of several traditional medicinal systems is more holistic. The fundamental mechanisms of these medicinal systems are still unexplainable using modern tools. The medicinal preparations in traditional medicines contain a variety of herbal and non-herbal ingredients that are thought to act on a variety of targets by various modes and mechanisms.

Based upon ethanopharmacological survey, the aim of the present work is to evaluate the anti-hyperglycemic and anti-oxidant activities of *Lantana camara* Leaves by using streptozotocin model in rats.

5. PLAN OF THE WORK

1. Selection of plant
2. Collection and authentification of the plant.
3. Extraction of *Lantana camara* Leaves with petroleum ether and 50% ethanol.
4. Preliminary phytochemical screening of different extracts of *Lantana camara* Leaves
5. Toxicological studies of *Lantana camara* Leaves
6. Anti-hyperglycemic activity of *Lantana camara* Leaves in streptozotocin induced diabetic rats.
7. Anti-oxidant activity of *Lantana camara* Leaves in streptozotocin induced diabetic rats.

6. MATERIALS AND METHODS

6.1 SELECTION OF PLANT

The selection of a medicinal plant for an anti-hyperglycemic activity, undergo discussion with a tribal medical practitioner for the traditional and tribal uses of the leaves of *Lantana camara which* have not been evaluated for anti-hyperglycemic activity. The compiled list of plants must therefore be subjected to a literature survey to confirm that the plant has not been previously investigated for anti-hyperglycemic activity. The plant have the glycosides which is used as the anti-hyperglycemic Hence the present work involves pharmacological evaluation such as anti-hyperglycemic, antioxidant activities, in addition to literature survey of the plant *Lantana camara.*

6.2 COLLECTION AND AUTHENTIFICATION OF THE PLANT

The leaves of *Lantana camara* (Family -Verbenaceae) were collected from Attur, Tamilnadu, in month of December 2016. The plant material was authenticated by Dr. M.Palanisamy, Scientist D In-charge, Botanical Survey of India, Southern regional Centre, Coimbatore, Tamilnadu - 641003 and the voucher specimen number BSI/SRC/5/23/2016/Tech/1211.

6.3 PREPARATION OF CRUDE DRUG FOR EXTRACTION

The authenticated leaves were used for the preparation of the extract. The roots was collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powdered was passed through sieve no. 40 and stored in an airtight container for extraction.

Method of extraction: Cold percolation process.

Requirements: Percolater, Shade dried coarse powder of leaves of Lantana *camara*

Solvents: Petroleum ether and 50% ethanol.

Methods

6.3.1 Preparation of petroleum ether extract of *Lantana camara*.

The powdered plant material (1000g) was macerated with petroleum ether to remove fatty substances and filtered by using whattman filter paper no. 1. The filtrate is dried under hot air oven. The residue was then stored in a desiccator.

6.3.2 Preparation of 50% ethanolic extract of *Lantana camara*.

The marc left was dried and then further exhaustively extracted with of 50% ethanol for 3 days (3 X 5L). The extract was separated by filtration and concentrated heating mantle until solid residue (yield 9.5 % w/w). The extract obtained was further subjected to toxicological and pharmacological investigations.

The percentage yield of the above extracts were expressed in (Table no. 3).

6.4 PRELIMINARY PHYTOCHEMICAL SCREENING

50% ethanolic extract of *Lantana camara* was subjected to qualitative tests for the identification of various active constituents' viz. carbohydrate, glycosides, alkaloids, amino acids, flavanoids, fixed oil, tannins, gum and mucilage, phytosterols etc. according to Khandelwal[76].

6.4.1 Test for carbohydrates and glycosides

A small quantity of the extract was dissolved separately in 4 ml of distilled water and filtered. The filtrate was subjected to the following tests to detect the presence of Carbohydrate and glycosides.

(a) Molisch's test

The filtrate was treated with 2-3 drops of 1% alcoholic α-napthol solution and 2 ml of concentrated H_2SO_4 was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

(b) Fehling's test

The filtrate was treated with 1 ml of Fehling's solution A and B and heated on the water bath. A reddish precipitate was obtained shows the presence of carbohydrate.

6.4.2. Test for fixed oils and fates

(a) Spot test

Small quantity of extract was pressed between two filter papers. Appearance of oil stain on the paper indicates the presence of fixed oil.

(b) Saponification test

Few drops of 0.5% alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on the water bath for 1-2 hours. Formation of soap or partial neutralization of alkali indicates the presence of fixed oils and fats.

6.4.3 Test for proteins and free amino acid

Small quantity of the extract was dissolved in few ml of distilled water and treated with following reagents.

(a) Millon's test

Appearance of red color shows the presence of proteins and free amino acids.

(b) Ninhydrin reagent

Appearance of purple color shows the presence of proteins and free amino acids.

(c) Biuret test

Equal volumes of 5% sodium hydroxide solution and 1% copper sulphate solution were added, appearance of pink or purple color shows the presence of proteins and free amino acids.

6.4.4 Test for saponins

(a) Foam test

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

6.4.5 Test for phenolic compounds

Small quantity of the extract was taken in distilled water and test for the presence of phenolic compounds and tannins was carried out with the following reagents.

(a) Dilute ferric chloride solution (5% w/v) - Violet color.

(b) 1% solution of gelatin containing 10% sodium chloride-White precipitate.

(c) 10% lead acetate solution-White precipitate.

6.4.6 Test for phytosterols

Small quantity of the extract was dissolved in 5 ml of chloroform separately. Then this chloroform solution was subjected to the following tests to detect the presence of phytosteroles.

(a) Libermann-Burchard's test

The above preapared chloroform solution was treated with few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3

ml of acetic anhydride. A bluish green color appeared indicates the presence of phytosterols.

(b) Salkowski reaction

To 1 ml of the above prepared chloroform solution, few drops of concentrated sulphuric acid was added. Brown color produced shows the presence of phytosterols.

6.4.7 Test for Alkaloids

Small quantity of the extract was treated with few drops of diluted hydrochloric acid and filtered. The filtrate was used for the following tests.

(a) Mayer's reagent – cream precipitate

(b) Dragendroff's reagent – Orange brown precipitate

(c) Hager's test – yellow precipitate

(d) Wagner's test – Reddish brown precipitate

6.4.8 Test for flavonoids

(a) With aqueous NaOH solution

Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow colour indicates the presence of flavonoids.

(b) With conc. sulphuric acid

To a small portion of extract, concentrated sulphuric acid was added. Yellow orange color was obtained shows the presence of flavonoids.

(c) Shinoda's test

Small quantity of extract was dissolved in alcohol; to those pieces of magnesium followed by concentrated hydrochloric acid was added drop by wise and heated. Appearance of magenta color shows the presence of flavonoids.

The results were shown in (Table no. 4).

6.5 PHARMACOLOGICAL STUDIES

6.5.1 Animals

Sprague-Dawley rats (150-185g) and Swiss albino mice (20-25 gm) of either sex and of approximately the same age were procured from the animal house of J.K.K.Nattraja College of Pharmacy, Kumarapalayam. They were kept in the departmental animal house at 26 ± 2 °C and relative humidity 44 – 56 % in polypropylene cages. The animals were exposed to alternate 12 hrs of darkness and light each. .Animals were provided with standard rodent pellet diet (Dayal, India) and the food was withdrawn 18-24 h before the experiment though water was allowed *ad libitum*. All experiments were performed in the morning according to current guidelines for investigation of experimental pain in conscious animals[77]. The standard orogastric cannula was used for oral drug administration in experimental animals.

6.6 EVALUATION OF TOXICOLOGICAL STUDIES OF *LANTANA CAMARA*

6.6.1 Acute toxicity studies (OECD Guideline 423)

Acute Toxic Category Method is a method for assessing acute oral toxicity that involves the identification of a dose level that causes mortality.

This test involves the administration of a simple bolus dose of test substances to faster healthy young adult rodents by oral gavage, observation for upto 15days after dosing and recording of body weight and the necropsy of all the animals. In this method pre-specified fixed doses of the test substances were used ie, 5mg/Kg, 50mg/Kg, 300mg/Kg, 2000mg/Kg and the mortality due to these doses were observed. Generally female animals were used for this study and each dose group should consist of 3 animals.

Requirements

Animal: Swiss albino mice (female 20-25 gm).

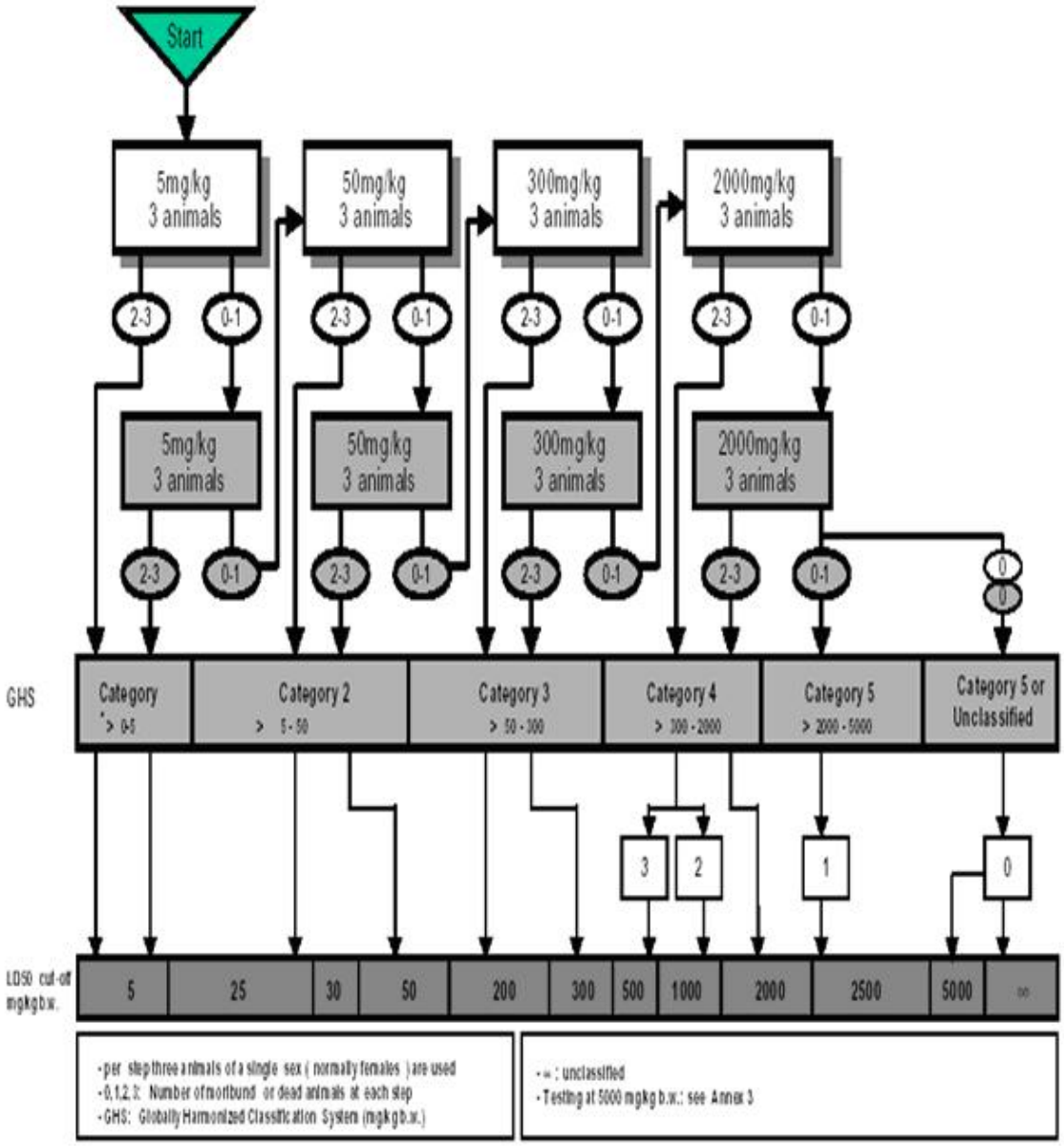
Drugs : 50% ethanolic extracts of *Lantana camara* Leaves.

Methodology

- The overnight fasted mice were weighed and selected.
- Ethanolic extract was dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity and were observed for a period of two weeks.
- The toxic doses were selected based on the Guideline 423

The results of the LD_{50} study was done by mice using guideline 423 method

TEST PROCEDURE WITH A STARTING DOSE OF 5 MG/KG BODY WEIGHT



6.7 EVALUATION OF ANTIHYPERGLYCEMIC ACTIVITY OF *LANTANA CAMARA* LEAVES IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

6.7.2 IN VIVO ANTI-HYERGLYCEMIC ACTIVITY

6.7.2.1 Experimental induction of diabetes

All animals were allowed to adapt to metabolic cages for 3 days, after which they were fasted overnight and 150 mg/kg streptozotocin (Sigma, St. Louis, MO, USA) freshly dissolved in normal saline was injected intraperitoneally. After streptozotocin treatment, all animals were returned to their cages and given free access to food and water. Blood glucose levels were measured 3 days after streptozotocin injection and used as parameter to obtain matching pairs of rats with diabetes of similar level of severity. Only rats with fasting blood glucose levels greater than 200 mg/dL were considered diabetic and then included in this study. The mean blood concentration of glucose in normoglycemic rats was 95 mg/dL. Diabetic rats were randomly assigned to four different groups (n = 6 animal/groups). All treatments started 3 days after streptozotocin injection[78].

6.7.2.2.1 Experimental Design

Group I	-	Control rats received vehicle solution (1% carboxy methyl cellulose)
Group II	-	Diabetic control rats received vehicle solution (1% carboxy methyl cellulose)
Group III	-	Diabetic rats treated with extract 200 mg/kg body weight in 1% carboxy methyl cellulose
Group IV	-	Diabetic rats treated with extract 400 mg/kg body weight in 1% carboxy methyl cellulose
Group V	-	Diabetic rats treated with Glibenclamide 5 mg/kg body weight in aqueous solution

The vehicles and the drugs were administered orally using intra gastric tube daily for three weeks. After three weeks of treatment the rats were fasted overnight, the blood samples were analyzed for blood glucose content. Then the animal was sacrificed by cervical decapitation. The liver, kidney and pancreas was exposed and perfused with cold phosphate buffer saline of pH 7.4. Blood free liver and kidney were taken out and homogenized in a glass Teflon homogeniser separately (10% w/v). Incubations were done at 37ºC under controlled conditions for biochemical estimations. Fresh blood drawn was centrifuged for 10 min at 2000 rpm. Erythrocyte sediment is resuspended twice in physiological NaCl solution (1:10) and centrifuged again in same solution. 250 µl of washed erythrocytes are then resuspended in 1000 µl physiological NaCl solution and stored at 4 °C in the dark until SOD measurement.

6.8 BIO-CHEMICAL PARAMETERS

6.8.1 Total cholesterol estimation

The serum cholesterol level was estimated by wybenga and pileggi method using cholesterol diagnostic reagent kit (span)

Principle

Cholesterol reacts with hot solution of ferric perchlorate, ethyl acetate and sulphuric acid (cholesterol reagent) and gives a lavender coloured complex which is measured at 560 nm.

Reagents

Reagent 1: Cholesterol reagent

Reagent 2: Working cholesterol standard, 200mg %

Procedure

Pipette into tubes marked	Blank	Standard	Test
Reagent 1 : Cholesterol reagent	5.0 ml	5.0 ml	5.0 ml
Reagent2 : Working cholesterol standard, 200mg %	-	0.025 ml	-
Serum	-	-	0.025 ml

Reagents were Mixed well and kept in the boiling water bath exactly for 90 seconds. Mixer was allowed to cool to room temperature under running tap water. Absorbance was measured at 560 nm.

Calculations

$$\text{Total cholesterol (mg/dl)} = \frac{\text{O.D. of test}}{\text{O.D. of standard}} \times 200$$

We can also measure the change of optical density directly from Bio Chemical analyzer at 560 nm were given in (Table no 7).

6.8.2 Triglycerides estimation

The triglycerides level was estimated by Glycerol phosphate oxidase (GPO) method.

Principle

Triglycerides in samples are hydrolyzed by microbial lipases to glycerol and free fatty acids(FFA).Glycerol is phasphorylated by adenosine-5-triphosphate (ATP) to glycerol-3-phosphate (G-3-P) in reaction catalyzed by glycerol kinase(GK).G-3-P is oxidized to dihydroxyacetone phosctphate (DAP) in reaction catalyzed by the enzyme glycerol phosphate oxidase (GPO).In this reaction hydrogen peroxide (H_2O_2) is produced in equimolar conc. to the level of triglyceride conc. in the sample.H_2O_2 reacts with 4-aminoantipyrine (4- AAP) and 3,5 dicholoro 2 hydroxy benzo sulfonic acid (DHBS) in a reaction catalyzed by peroxidase (HPOD).The result of this oxidative coupling is a quinoemine red colour dye.

Reaction

$$\text{Triglycerides} \xrightarrow{\text{Lipases}} \text{glycerol + free fatty acid}$$

$$\text{Glycerol + ATP} \xrightarrow{\text{GK}} \text{G-3-P + ADP } Mg{+}2$$

$$\text{G-3-P + } O_2 \xrightarrow{\text{GPO}} \text{DAP + } H_2O_2$$

HPOD

$2H_2O_2$ + 4 AAP +DHBS ⟶ Red Quinoneimine dye + $4H_2O$

Requirements

Enzyme Reagent	3 ml
Buffer	30 ml
Triglyceride Standard	3 ml

Procedure

Bring required reagent and samples to 37 ◦C before performing the assay

Reagents	Test	Standard	Blank
Working Reagent	1.0 ml	1.0 ml	1.0 ml
Sample	10µl	----	----
Standard	----	10µl	----

Mix and Incubate at 37 ^{o} C for 10 min. Read the absorbance against reagent blank on a spectrophotometer at 520 nm against reagent blank. The final colour stable for 30 min.

Calculation

$$\text{Triglyceride conc. (mg / dl)} = \frac{\text{A of Test}}{\text{A of Std.}} \times 200$$

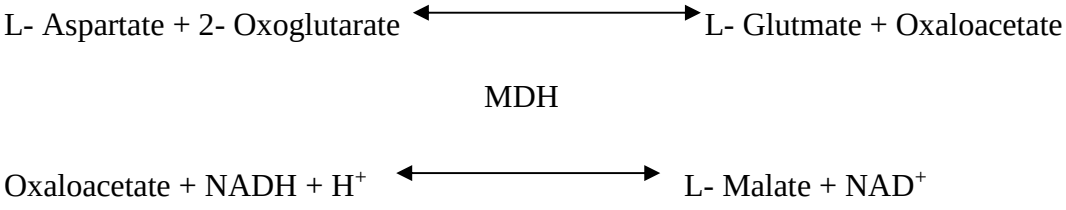
We can also measure the change of optical density directly from Bio Chemical analyzer at 520 nm were given in (Table no 7).

Serum was analyzed for the following parameters aspartate aminotransferase/serum glutamic oxaloacetic transaminase (ASAT)/(SGOT), alanine amino transferase/serum glutamate pyruvate transaminase (ALAT)/(SGPT), alkaline phosphatase (ALP) and cholesterol were given in (Table no 8).

6.8.3 Determination of Serum glutamic oxaloacetic transaminase (SGOT)

The SGOT activity was determined according to the method of IFCC modified method using SGOT (Liquizone diagnostic reagent kit).

Principle



Reagents

R1 : TRIS- pH 7.65,L- Aspartate, MDH (Malate dehydrogenase), LDH (Lactate dehydrogenase)

R2: Oxoglutarate, NADH

Mix 1 part of R2 with 4 parts of R1 as required

Procedure

Working Reagents	1.0 ml
Serum / Plasma	100 µl

Mix well and after 1 minute incubation, measure the change of optical density per 60 sec during 180 sec against distilled water at 340 nm as follows.

A0 - Exactly after 1 min

A1, A2, A3 - Exactly after every 60 sec for 180 sec

Calculations:

Calculate the average change in absorbance per minute (Δ Abs/min).Activity of ASAT (SGOT) in IU/L

At 340 nm in IU/L = Δ Abs/min × 1746 × tf

Temperature conversion factors (tf):

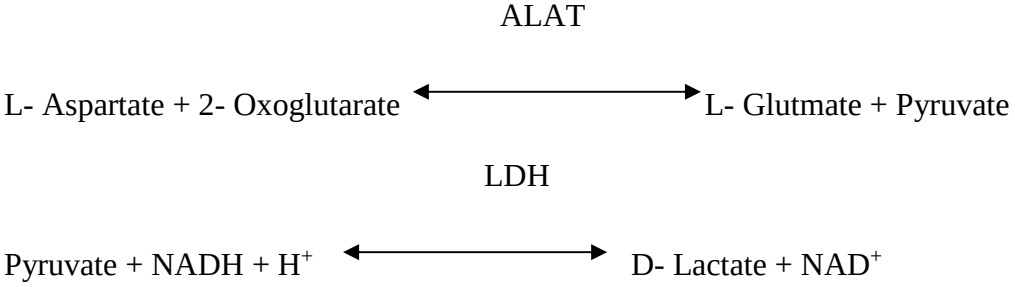
Assay Temperature	Temperature factor (tf)
25◦C	2.08
30◦C	1.54
37◦C	1.00

We can also measure the change of optical density directly from Bio Chemical analyzer at 340 nm were given in (Table no 8).

6.8.4 Determination of Serum glutamate pyruvate transaminase (SGPT) or ALAT

The SGPT activity was determined according to the method of IFCC modified method using SGPT (Liquizone diagnostic reagent kit).

Principle



Reagents

R1: TRIS- pH 7.15, L- Alanine, LDH (Lactate dehydrogenase)

R2: Oxoglutarate, NADH

Mix 1 part of R2 with 4 parts of R1 as required

Procedure

Working Reagents	1.0 ml
Serum / Plasma	100 µl

Mix well and after 1 minute incubation, measure the change of optical density per 60 sec during 180 sec against distilled water at 340 nm as follows.

A0 - Exactly after 1 min

A1, A2, A3 - Exactly after every 60 sec for 180 sec

Calculations:

Calculate the average change in absorbance per minute (Δ Abs/min).Activity of ALAT (SGPT) in IU/L

At 340 nm in IU/L = Δ Abs/min × 1746 × tf

Temperature conversion factors (tf):

Assay Temperature	Temperature factor (tf)
25◦C	1.82
30◦C	1.39
37◦C	1.00

We can also measure the change of optical density directly from Bio Chemical analyzer at 340 nm were given in (Table no 8).

6.8.5 Determination of serum alkaline phosphatase (SALP)

The alkaline phosphates level was estimated by p- Nitrophenyl phosphate (PNPP) method (Qualigens diagnostic reagent kit).

Principle

The determination of the activity of alkaline phosphatase in serum based on the hydrolysis of p- nitrophenyl phosphate (PNPP) by the enzyme with the formation of free p- nitrophenol.This compound is yellow in alkaline solution. The formation of yellow colour can be spectrophotometrically readapt 405 nm, which is directly proportional to the enzymatic activity of alkaline phosphatase in serum / plasma.

$$PNPP + H_2O \xrightarrow{\text{Alkaline Phosphatase}} \text{P- nitrophenol} + \text{Phosphate}$$

The method has been recommended by the German Society of clinical chemistry and by the committee on enzyme of the Scandinavian Society of Clinical Chemistry and Clinical Physiology.

Reagents

Reagents 1: Substrate

Reagents 2: Buffer

Preparation of working solution

Dissolve each vial content (Reagent 1) of dry substance with 3.0 ml of buffer (Reagent 2). Mix to dissolve by slow stirring to ensure uniform mixing.

Procedure

	Test(T)	Blank(B)
Working reagent	1.0 ml	Distilled water
Sample	20 µl	Distilled water

Mix well and read the absorbance at 60, 90, 120 and 150 sec at 405 nm. Determine the Δ A /min from the linear part of the assay.

Calculation

IU /L of Alkaline phosphatase = Δ A /min × 2713

Where F = 2713 is calculated on the basis of molar extinction coefficient for p-nitrophenol and total assay volume to sample volume.

We can also measure the change of optical density directly from Bio Chemical analyzer at 405 nm were given in (Table no 8).

6.9 EVALUATION OF ANTIOXIDANT ACTIVITY OF *LANTANA CAMARA* IN STREPTOZOTOCIN INDUCED DIABETIC RATS.

6.9.1 Assay of lipid peroxidation

The concentration of thiobarbituric acid reactive substances (TBARS) was measured (lipid peroxidation product maondialdehyde (MDA) was estimated) in liver using the method of Okhawa et al[79]. 1 ml of the sample was mixed with 0.2 ml 4 % (w/v) sodium dodecyl sulfate, 1.5 ml 20% acetic acid in 0.27 M hydrochloric acid (pH 3.5) and 15 ml of 0.8% thiobarbituric acid (TBA, pH 7.4). The mixture was heated in a hot water bath at 85˚C for 1 h. The intensity of the pink colour developed was read against a reagent blank at 532 nm following centrifugation at 1200 rpm for 10 min. The concentration was expressed as n moles of MDA per mg of protein using 1,1, 3,3,-tetra-ethoxypropane as the standard.

The results were given in (Table no. 9).

6.9.2 Catalase activity

CAT activity was determined by monitoring the enzyme-catalyzed decomposition if hydrogen peroxide by potassium permanganate according to Cohen et al.[80] Samples (50 µl) were added to test tubes followed by the addition of H_2O_2, then allowed to incubate on ice for 3 min. The reaction was stopped by the addition of H_2SO_4. $KMnO_4$ was then added and absorbance was recorded at 480 nm. Under the conditions of this assay, one unit of enzyme activity equals k/(0.0693) according to Aebi[81], where k = log (S_0/S_2) x (2.3/t), S_0 = absorbance of standard – absorbance of blank, S_2 = absorbance of standard – absorbance of sample, and t = time interval. The measured activities were normalized to the protein content of each sample.

The results were given in (Table no. 9).

6.9.3 Superoxide dismutase activity

SOD was estimated using the standard method of Kakkar et al.[82] Assay mixture contained 1.2 ml sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml 186µM phenazine methosulphate, 0.3 ml 300 µM nitroblue tetrazolium, 0.2 ml NADH (780 µM), appropriately dilute enzyme preparation and water in a total

volume of 3 ml. Reaction was started by the addition of NADH. After incubation at 30 °C for 90 secs, the reaction was stopped by the addition of 1 ml glacial acetic acid. Reaction mixture was stirred vigorously and shaken with 4 ml of n-butanol. The mixture was allowed to stand for 10 min, centrifuged and butanol layer taken out. Colour intensity of the chromogen in the butanol was measured at 560 nm. A system devoid of enzymes served as control. One unit of the enzyme activity is defined as the enzyme concentration required to inhibit the optical density at 560 nm of chromogen production by 50 % in one min under the assay conditions and expressed as specific activity in milliunits/ mg protein.

The results were given in (Table no. 9).

6.9.4 Glutathione peroxidase

The incubation mixture at 37 ° C contained 0.08 M sodium phosphate (pH 7.0), 0.08 M EDTA, 1.0 mM sodium azide, 0.4 nM GSH and 0.25 mM H_2O_2. GSH was determined at 3 minute intervals using DTNB[83]. An enzyme unit represents a decrease in GSH concentration of 0.001 log unit per minute after subtraction of nonenzymic mode[84].

The 50% ethanolic extracts of leaves of *Lantana camara* and glibenclamide treated rats showed decreased LPO that is associated with increased activity of SOD, catalase and GPx were given in (Table no. 9).

6.10 STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM (Standard Error Mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 3.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Newman-Keuls Multiple Comparision Test.

The value of probability less than 5% ($P < 0.05$) was considered statistically significant.

7. RESULTS

7.1. Preliminary phytochemical screening

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents in 50% ethanolic extract of *Lantana camara in* (Table 4) and percentage yield in (Table 3).

7.2. Pharmacological Investigations

7.2.1. General behavior and acute toxicity studies

50% ethanolic extract of selected plant *Lantana camara* Leaves *up* to 2000 mg/kg did not cause any mortality in mice. None of the doses tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output, feeding behavior etc. during the period of observation.

7.2.2. Effect of 50% ethanolic extract of *Lantana camara* on STZ induced diabetes

7.2.2.1. Effect of 50% ethanolic extract of Lantana camara on STZ induced diabetic rats after 0 day and 21 days

STZ induced the significant increase in the blood glucose level at 0 day (72.33-292.66, $p<0.0001$). The 50% ethanolic extract of the *Lantana camara* showed the significant effect compared with the respective diabetic control group, decrease the blood glucose level at a dose of 200 mg/kg and 400 mg/kg (274.75-205.05, 274.75-199.83,$p<0.0001$), the standard drug glibenclamide 5mg/kg also showed the significant decrease the blood glucose level after 21 days (274.75-162.16, $p<0.0001$). Finally the 400 mg/kg and the standard drug showed the significant decrease in the blood glucose level after 21 days treatment ($p<0.0001$) as given in (Table 5).

7.2.2.2. Effects of 50% ethanolic extract of Lantana camara on the blood glucose Levels of STZ induced diabetic rats

The blood glucose levels of diabetic rats treated with LCE at doses of 200 and 400 mg/kg showed significant differences at 2, 3 and 4 h from initial levels ($P < 0.0001$). Only diabetic rats with blood glucose level of 252.0 ± 1.59 mg/dl showed a significant and pronounced reduction of glycemia ($P < 0.0001$) 2, 3 and 4 h after oral glibenclamide (5 mg/kg) administration. The LCE was found to be slow and less effective than glibenclamide in diabetic rats as given in (Table 6).

7.2.2.3. Effect of 50% ethanolic extract of Lantana camara on cholesterol and Triglyceride, in STZ induced diabetic rat serum

It is clearly evident that STZ caused significant elevation of serum markers. The STZ treated group, the level of cholesterol (72.82-103.61, p<0.0001), triglyceride (81.25-105.79, p<0.0001). In contrast, the groups treated with *Lantana camara* Leaves extract at dose 200 and 400mg/kg once daily for 21 days prevented the diabetes condition in a dose related manner. The range of protection were found to be, cholesterol (103.61-79.54, p<0.05, p<0.0001), triglyceride (105.79-84.01, p<0.05, p<0.0001) and glibenclamide (5 mg/kg) also showed the significantly decrease with respect to diabetic control group (103.61-76.71, p<0.0001, p<0.01, 105.79-84.01, p<0.0001,p<0.05) (Table 7).

7.2.2.4. Effect of 50% ethanolic extract of Lantana camara on ***SGOT, SGPT and SALP***

STZ induced the significant increase in SGOT(63.59–73.73,p<0.01,p<0.0001),SGPT(21.28-29.56,p<0.05,p<0.0001) and SALP(229.66-261.5,p<0.0001).The 50% ethanolic extracts of 200, and 400 mg/kg of *Lantana camara* decreases the SGOT (73.73-67.90,p<0.0001,p<0.01,p<0.05), SGPT (29.56-25.12,p<0.0001,p<0.05) and SALP (261.5-234.66,p<0.05,p<0.0001) levels with respect to diabetic control group and glibenclamide (5 mg/kg) also showed

the significantly decrease with respect to diabetic control group (73.73-65.92,29.56-23.16,261.5-232.33,p<0.0001,p<0.05)(Table 8).

7.2.2.5. Effect of 50% ethanolic extract of Lantana camara on LPO, CAT, SOD, GPx against the STZ induced diabetic rats

STZ caused significant elevation in LPO (0.31-0.46, p<0.001) compared with respective control group, the 50% ethanolic extract of *Lantana camara* decrease the LPO level (0.46-0.39, p<0.01, p<0.05, p<0.001) and standard drug glibenclamide decrease significantly the LPO level(0.46-0.36).The SOD, CAT, GPx significantly decreased by STZ (145.70-128.39, 44.57-27.26, 7.05-2.97), 50% ethanolic extract of *Lantana camara* increased the level of SOD (128.39-139.85,p<0.0001), CAT (27.26-35.14, p<0.01, p<0.0001), GPx (2.95-4.55,p<0.01, p<0.001). Standard drug glibenclamide increased significantly SOD (128.39-142.40, p<0.001), CAT (27.26-39.31, p<0.001), GPx (2.95-5.71, p<0.001) (Table 9).

Table 3. Percentage yield of *Lantana camara* with 50% ethanol

Plant used	Part used	Method	Percentage yield
Lantana camara	Leaves	Percolation with 50% ethanol	9.5% w/w

Table 4. Preliminary phytochemical screening of the 50 % ethanolic extract of *Lantana camara* (LCE)

S. No.	Constituents	Tests	50% Ethanolic extract
1.	**Carbohydrate &Glycosides**	Molish's test	+
		Fehling's test	+
2.	**Fixed oil & fats**	Spot test	+
		Saponification test	+
4.	**Proteins & amino acids**	Million's test	_
		Ninhydrin test	_
		Biuret test	_
5.	**Saponins**	Foam test	+
6.	**Phenolic compounds**	$FeCl_3$ test	+
		Gelatin test	_
		Lead acetate test	+
7.	**Phytosterol**	Salkowiski test	+
		Libermann burchard test	+
8.	**Alkaloids**	Dragendroff's test	+
		Mayer's test	+
		Wagner's test	+
		Hager's test	_
9.	**Gum & mucilage**	Swelling test	−
10.	**Flavonoids**	Aqueous NaOH test	+
		Con. H_2SO_4 test	+
		Shinoda's test	+

Where,

+ = Presence

− = Absence

Table 5. Anti-diabetic effect (Glucose level) of 50% ethanolic extract of *Lantana camara* (LCE) on STZ induced diabetic rats after 21 days.

Groups	0 day (mg/dl)	After 21 days (mg/dl)
Control	72.33±0.71	72.66 ± 0.88
Diabetic control	292.66 ± 1.14	274.75± 1.43
LCE (200 mg/kg)	280.16 ±0.94	205.5[b]± 1.73
LCE (400 mg/kg)	272.33± 0.66	199.83± 1.22
Glibenclamide (5 mg/kg)	252.00 ± 1.59	162.16 ± 1.35
One-way ANOVA F df p	7,411 4 <0.0001	2,739 4 <0.0001

Value are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group

b = P < 0.01 – (LCE 400 Vs. LCE 200)

Table 6. Effect of LCE on serum glucose level in STZ induced diabetic rats

Groups	0 hr. (mg/dl)	2 hr. (mg/dl)	3 hr. (mg/dl)	4 hr. (mg/dl)
Normal control	72.33±0.71	71.83 ± 0.94	72.16 ± 0.40	71.66 ± 0.55
LCE(200 mg/kg)	280.16 ± 0.94	276.0 ± 0.57	274.66 ± 0.71	270.16 ± 0.75
LCE(400 mg/kg)	272.33 ± 0.66	267.83 ± 0.60	264.66 ± 0.80	260.83 ± 0.91
Glibenclamide (5 mg/kg)	252.0 ± 1.59	248.16 ± 0.98	246.16 ± 0.75	241.66 ± 1.28
One-way ANOVA				
F	8,943	14,820	19,570	10,540
df	3	3	3	3
p	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Values are expressed as Mean ± SEM of 6 rats in each group.

Table 7. Effect of 50% ethanolic extract of *Lantana camara* (LCE) on cholesterol, and triglyceride levels in blood serum

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Control	72.82 ± 1.01	81.25 ± 0.97
Diabetic control	103.61±0.43	105.79± 1.66
LCE (200 mg/kg)	82.25[a]± 0.97	99.95 ± 0.69
LCE (400 mg/kg)	79.54[a] ± 0.94	88.99 ± 0.70
Glibenclamide (5 mg/kg)	76.71[b] ±0.76	84.01[a] ± 0.97
One-way ANOVA **F** **df** **p**	 140.9 4 <0.0001	 109.9 4 <0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a= P< 0.05- (Std. Vs. LCE 400, LCE 400 Vs. LCE 200, Cont. Vs. Std

b = P < 0.01- (Cont. Vs. Std)

Table 8. Effect of 50% ethanolic extract of *Lantana camara* (LCE) on SGOT, SGPT, and SALP level in blood serum

Oral treatment (mg/kg, OD x 21 days)	SGOT (U/l)	SGPT (U/l)	SALP (U/l)
Control	63.59 ± 0.55	21.28 ± 0.29	229.66 ± 0.76
Diabetic control	73.73^{b} ± 0.53	29.56^{a} ± 0.79	261.5 ± 1.04
LCE (200 mg/kg)	70.91^{b} ± 0.53	27.10 ± 0.47	237.16^{a} ± 0.60
LCE (400 mg/kg)	67.90^{a} ± 0.85	25.12^{a} ± 0.76	234.66^{a}± 0.55
Glibenclamide (5 mg/kg)	65.92^{a} ± 0.33	23.16^{a} ± 0.87	232.33^{a} ± 0.71
One-way ANOVA			
F	39.45	22.17	253.8
df	4	4	4
p	< 0.0001	< 0.0001	< 0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a= P< 0.05 - (Cont. Vs. Std., Std. Vs. LCE 400), (Cont. Vs. Std., Std. Vs. LCE 400, LCE 400 Vs. LCE 200, LCE 200 Vs. Dia. Control.), (Cont. Vs. Std., Std. Vs. LCE 400, LCE 400 Vs. LCE 200)

b = P < 0.01- (LCE 400 Vs. LCE 200, LCE 200 Vs. Dia. Control)

The differences in mean SGOT, SGPT, SALP, in extract treated groups are not significantly different from control group at the end of study (21 days)

Table 9. Effect of 50% ethanolic extract of *Lantana camara* (LCE) on lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase in 21 days

Oral treatment	LPO (n moles/mg of protein)	SOD (units/mg of protein)	CAT (units/mg of protein)	GPx (m moles/gm.)
Control	0.31 ± 0.006	145.70[b] ± 0.42	44.51 ± 0.81	7.05 ± 0.09
Diabetic control	0.46[b] ± 0.008	128.39 ± 1.65	27.26 ± 1.10	2.95 ± 0.07
LCE (200 mg/kg)	0.42[a] ± 0.014	134.18 ± 0.66	31.51[b] ± 1.12	3.66[b] ± 0.07
LCE (400 mg/kg)	0.39[a] ± 0.009	139.85 ± 0.51	35.14[a] ± 0.83	4.55 ± 0.08
Glibenclamide (5 mg/kg)	0.36 ± 0.004	142.40[a] ± 0.88	39.31[b] ± 0.94	5.71 ± 0.25
One-way ANOVA				
F	36.42	65.27	44.97	129.9
df	4	4	4	4
p	< 0.0001	<0.0001	< 0.0001	<0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a= P< 0.05 - (Std. Vs. LCE 400, LCE 400 Vs. LCE 200), (LCE 400 Vs. Std.), (LCE 200 Vs. LCE 400)

b = P < 0.01- (LCE 200 Vs. Dia. control), (Std. Vs. Control), (Dia. control Vs. LCE 200, LCE 400 Vs. Std.), (Dia. control Vs. LCE 200)

8. DISCUSSION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin[85]. The number of people in the world with diabetes has increased dramatically over recent years. Indeed, by 2010 it has been estimated that the diabetic population will increase to 221 million around the world[86].

Present number of diabetics worldwide is 150 million and this is likely to increase to 300 million or more by the year 2025[87]. Reasons for this rise include increase in sedentary lifestyle, consumption of energy rich diet, obesity, higher life span, etc.[88]. Regions with greatest potential are Asia and Africa, where diabetes mellitus (DM) rates could rise to 2–3-folds than the present rates[89.] Many herbal medicines have been recommended for the treatment of diabetes[90]. Plant drugs are frequently considered to be less toxic and freer from side effects than synthetic ones[91]. STZ induced diabetes cause an increase in blood glucose level in rats[92]. Our studies show that oral administration of 50 % ethanolic *Lantana camara* Leaves extract decreases blood glucose in diabetic rats. The increase in the level of lipid peroxides in plasma generally is thought to be the consequence of increased production of and liberation in to the circulation of tissue lipid peroxides due to pathological changes[93]. This action shows the anti-peroxidative effect of LCE. Changes in the levels of antioxidants are observed in diabetic conditions[94].

Free radical-induced LPO has been associated with a number of disease processes including diabetes mellitus[95]. The increase in oxygen-free radicals in diabetes could be due to increase in blood glucose levels, which generate free radicals upon autoxidation[96]. Glucose auto-oxidases in the presence of transition metal ions generating oxygen-free radicals which make the membrane vulnerable to oxidative damage. The action of diabetes-inducing agents produces reactive free radicals, which have been shown to be cytotoxic to the ß cells of the pancreas[97] .The diabetogenic action can be prevented by the superoxide dismutase, catalase and other hydroxyl radical scavengers, such as ethanol and dimethyl urea, hence there is evidence to suggest that the incidence of diabetes

involves superoxide anion and hydroxyl radicals. The deleterious effects of superoxide anion and hydroxyl radicals can be counteracted by antioxidant enzymes, such as SOD, CAT etc. There is clear cut evidence to show the role of free radicals in diabetes and studies indicate that tissue injury in diabetes may be due to free radicals[98]. The capacity of NAE to significantly decrease the elevated blood glucose close to normal level is an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes. These findings coincide with those of the earlier studies which report the anti-diabetic activity of the plant by clinical studies[99]. LPO is also one of the features of chronic diabetes and lipid peroxide-mediated damage has been observed in both type I and type II diabetes mellitus. Under physiological conditions, low concentrations of lipid peroxides are found in tissues, which stimulate the secretion of insulin[100] .The involvement of free radicals in diabetes and the role of these toxic species in LPO and the antioxidant defense system have been studied. Depletion of tissue glutathione and increase in LPO have been observed in diabetes.[101]

The present study demonstrated that the 50% ethanolic *Lantana camara* Leaves extract had an anti-hyperglycemic effect in the STZ induced diabetic rats when administered orally. Hypoinsulinemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase which initiates beta oxidation of fatty acids, resulting in lipidperoxidation[102]. Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors.[103] .Its products are harmful to most of the cells in the body and associated with variety of disease.[104]

SOD and CAT are the two scavenging enzymes that remove the toxic free radicals.[105] .In the enzymatic antioxidant defence system, SOD is one of the most important enzymes and scavenges O_2- anion (which is the first product of O2 radicals) to form H_2O_2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions.[106] .The O_2- anion is known to inactivate CAT and GPx [107]. Catalase has been regarded as a major determinant of hepatic and cardiac antioxidant status[105]. It is known to be involved in detoxification of H_2O_2 concentrations[108]. Whereas GPx is

sensitive to lower concentrations of H_2O_2. These enzyme activities were inactivated by ROS during diabetes[109].

One of the consequences of hyperglycemia is increased metabolism of glucose by sorbitol pathway. Besides this, other pathways, such as fatty acid and cholesterol biosynthesis also compete for NADPH with GSH. The decrease in GSH level in liver during diabetes is probably due to its increased utilization by the hepatic cells which could be the result of decreased synthesis or increased degradation of GSH by oxidative stress in diabetes[110]. The activities of GPx was observed to decrease significantly in diabetic rats. GPx, an enzyme with selenium and GST, catalyzes the reduction of hydrogen peroxide to non-toxic compounds[111]. Administration of LCE and glibenclamide increased the activities of GPx and GST in diabetic conditions. SOD and catalase are two major scavenging enzymes that remove the toxic-free radical in vivo. Reduced activities of SOD in erythrocytes and catalase in liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxide[112]. LCE and glibenclamide treated rats showed decreased LPO that is associated with increased activity of SOD and catalase. The results obtained thus suggest that 50% ethanolic *Lantana camara* Leaves extract possesses potent anti-hyperglycemic and antioxidant activity. It is hoped that the activity-guided isolation of the extract of this plant may yield valuable therapeutic compound(s) useful for developing powerful hypoglycemic or antioxidant drugs. The study also demonstrates that pharmacological screening based on the ethnomedical leads can yield faster hits in search of therapeutic agents from plants.

9. CONCLUSION

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources.

Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action. Herbal treatments for diabetes have been used in patients with insulin-dependent and non-insulin-dependant diabetes, diabetic retinopathy, diabetic peripheral neuropathy, etc. Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the sugar level. From the reports on their potential effectiveness against diabetes, it is assumed that the botanicals have a major role to play in the management of diabetes.

In recent year several authors evaluated and identified the anti-diabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus. Effect of 50 % aqueous alcoholic extract of plants on serum glucose, lipid profile and antioxidant status in streptozotocin induced diabetic rats was studied. Based on this, potentiation of dreaded disease like diabetes mellitus may shows a ray for better protocol for future treatment. The efficacy of *Lantana camara* Leaves in experiment showed the significant decrease in the blood glucose level, increase the antioxidant efficacy in streptozotocin induced diabetes. It was demonstrated that the oral administration of the 50% ethanolic *Lantana camara* Leaves extract to streptozotocin diabetic rats is useful for the treatment of diabetes induced by streptozotocin, because there were significant positive changes in the biochemical and physiological parameters related to carbohydrate, protein and lipids metabolism., the strong anti-hyperglycemic and antioxidant effect observed in STZ induced diabetic rats justifies the use of *Lantana camara* Leaves for the treatment of diabetes related complications.

The present study showed that the ethanolic extract of *Lantana camara* Leaves not only possess anti-hyperglycemic properties but also antioxidant activity in diabetic condition. These observation and description of mechanism of *Lantana camara* Leaves, which interplay with diabetes biology and pharmacology lead to rapid development in diabetes treatment. In addition to this, studies on molecular aspect of diabetic therapy will give mechanistic information in diabetes therapy and also critical balance should be there between the animal model and clinical research. This holds great promise for future research in human beings.

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